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The Fate of Arsenic in Soil-Plant Systems

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1 Introduction

1.1 The Properties of Arsenic

Arsenic (As) is an element belonging to the group V-A, and demonstrates characteristics of a metalloid. Because arsenic more easily forms anions, its non-metal properties dominate. When arsenic is in an oxidation state of +5, it acts similarly to phosphorus, a fact that has many implications for the way in which it reacts in soil, as well as its potential toxicity in plants. The most common oxidation states of As are -3, 0, +3, and +5. Arsines and metal arsines are those in which As has an oxidation state of -3, and these forms, are very unstable under oxidizing conditions. Under aerobic conditions, the oxidation state of As tends to be +5, and, when this occurs at a pH of between 2 and 3 the arsenic acid (H_3AsO_4) is formed. When the pH rises to values between 3 and 11, this compound disassociates to $H_2AsO_4^-$ and $HAsO_4^{2-}$ (Smedley and Kinninburgh 2002). Under anaerobic conditions, the predominant As species is H_3AsO_3. Arsenic also biomethlyates easily (Barán 1995).

Arsenic is widely distributed throughout the environment, and can be detected in the lithosphere in concentrations between 1.5 and 2 mg kg^{-1}, making it the 52^{nd} most abundant element (Adriano 2001). Arsenic forms a part of more than 245 minerals that include arsenates (60%), sulfides and sulfosals (20%), as well as other compounds such as arseniurates, arsenites, oxides and silicates (20%) (Mandal and Suzuki 2002). The majority of arsenic deposits in the earth's surface are found as sulfurous minerals (arsenopyrite).

1.2 The Presence of and Exposure to Arsenic in the Environment

Inorganic arsenic is present in soil, water, air and food such that humans are constantly exposed to this contaminant (Mandal and Suzuki 2002). The range at which arsenic is present in soils varies normally between 0.2 and 40 mg kg^{-1}, while in urban areas the concentration in atmospheric air is approximately 0.02 µg m^{-3}. On a global level, drinking contaminated water is the major source of exposure to this contaminant (Smedley and Kinninburgh 2002). It is estimated that some thirty million people are exposed to waters contaminated with arsenic, a quarter of them showing symptoms associated with long term exposure in at least five South Asian countries: Bangladesh, India, Nepal, Tailandia and Myanmar (Caussy 2003). The World Health Organization (WHO) recommends a maximum level of arsenic in waters of 10 µg L^{-1} (Bissen and Frimmel 2003); however, the concentration of arsenic in surface waters is greater than

2000 μg L^{-1} in certain regions of Bangladesh and India (Tripathi et al. 2007). Soil and water are the main sources of human exposure to arsenic at any given location, either by consumption (greatest in children; Rodríguez et al. 2003), inhalation or direct skin contact (DEFRA 2002). Because arsenic accumulates in vegetables, fruits, and other plants that grow in contaminated soils, another important pathway of exposure is the transfer of arsenic within the food chain (Meharg and Hartley-Whitaker 2002).

In terms of anthropogenic sources, annual global production of arsenic is estimated to be between 75 and $100 \cdot 10^3$ t (Adriano 2001). Natural sources (those in which human intervention does not play an important role) will depend, in many cases, on the geochemistry of each site, principally of the site's lithology and dispersion pathways. The major human activities that produce As are mining, metallurgy, agriculture, forestry, fossil fuel treatment plants, urban waste and cattle farming (Adriano 2001; Fitz and Wenzel 2002). In both mining and metallurgy, arsenic is produced as a by-product of little value, making it an unwanted waste. There are important arsenic deposits in some components of the earth's surface, which gives way to an elevated concentration of arsenic in the adjacent environment (up to 3% As in the soil) that rapidly decreases with distance from the contaminated sites (Zhang and Selim 2008). Also, some industrial activities may be a source of As; for example, when old glass manufacturing industries disposed of rich As wastes in the early 20th century (Madejón and Lepp 2007). Many biocides, used to control diseases in agriculture and forestry, are composed of As (Lepp 1981). Similarly, the use of fertilizers is also a source of arsenic in soils (Matschullat 2000). Urban wastes derived from treatment plants and compost, often used as organic amendments, may contain arsenic in varying quantities (Beesley and Dickinson 2010). Lastly, fossil fuel combustion also produces quantities of arsenic that may lead to long term accumulation from the gases emitted to the surrounding areas (Matschullat 2000). All of these factors release arsenic into the environment and can result in its accumulation in soils. When present in soils, As is generally observed to be more abundant in its inorganic form, with As(V) as the predominant species found under aerobic conditions (Akter and Naidu 2006). In soils, organic As species are usually found as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Takamatsu et al. 1982; Mestrot et al. 2011).

2 The Dynamics of Arsenic in Soil

Some authors believe that base concentrations of 10-40 mg kg^{-1} of arsenic exist in areas where the lithology has no known unnatural sources of contamination (Fitz and Wenzel 2002; Mandal and Suzuki 2002); however, estimates of the average concentration that exists in the pedosphere are only 5-8 mg kg^{-1} (Matschullat 2000). In the Andalusia region of Spain, values of 33 mg kg^{-1} of As have been documented to occur in soil, although amounts are highly dependent on the soil horizon, the type of soil and the lithology (Martín et al. 2007). In rare instances, either because of natural or anthropogenic sources, some soils are known to contain extraordinarily high levels of arsenic, i.e., values of 0.1 and 2% arsenic (Chang et al. 2005; Ongley et al. 2007; King et al. 2008). Such places pose a risk to human health as well as to ecosystem health, and if deemed necessary, these areas must be managed to reduce probable exposure risks.

An important variable in the study of As in soils is the heterogeneity in which it can appear, such that regions displaying high concentrations may be adjacent to regions that have much lower levels. In such cases, contaminant hot spots must be identified, because they will pose the hardest-to-manage threats (Dickinson et al. 2009). Several authors have described events in which As levels are greater in surface soil horizons (Adriano 2001; Clemente et al. 2008). When this occurs, it would indicate that contamination took place after the genesis of the soil in which it appears. However, this phenomenon depends on the source and method by which arsenic made its way into the soil. In one review (Fitz and Wenzel 2002), the authors described how, in the European Union, there may be up to 1.4 million soil sites that are contaminated with metals, metalloids, and/or organic compounds. These authors also estimated that, in the United States, approximately 41% of the soil sites catalogued as being contaminated were catalogued as such because of arsenic. Similarly, in Australia, there are more than 10,000 soil sites contaminated by As. Some of these Australian sites are extensive in area and constitute large-scale events of As contamination.

When setting safe levels, environmental legislation or regulation tends to rely on values that reflect total arsenic levels, but the total content of a trace element, as determined by acid digestion, is rarely a good indicator of the element's mobility or potential risk (Allen 2001).

2.1 The Mobility and Solubility of Arsenic in Soils

The solubility of soil contaminants is a key parameter to understanding their probable mobility. The soluble fraction in which contaminants exist is in equilibrium with others that exist in the soil environment. Once dissolved in soil water, elements are often present as different species that have different ionic activities (Sauvé 2001). Typical concentrations of arsenic in the soil solution, under aerobic conditions are <50 nM in non-contaminated soils, while they can reach values of up to 2 μM in contaminated soils (Wenzel et al. 2002; Moreno- Jiménez et al. 2011a); however, an exception was one sample from a semi-flooded mine soil that had up to 40 μM As. In flooded soils (where the predominate species is arsenite), typical concentrations of As in solution vary between 0.01 and 3 μM (Zhao et al. 2009). In one As study, using lupine plants as indicators of As availability, the behavior of the in-soil crop was compared with that same crop grown hydroponically. In this study, an exposure of less than 10 μM of As in soil solution was established, wherein the total concentration of As was more than 2000 mg kg^{-1} (Moreno-Jiménez et al. 2010b). In comparison to other trace elements, arsenic shows a low solubility in well-aerated soils (Beesley et al. 2010a; Moreno-Jiménez et al. 2010a).

Traditionally, sequential extraction has been used to fractionate trace elements that appear in soil. For arsenic, which is usually present in its anionic form in soils, specific protocols for arsenic extraction have been developed from these methods (Onken and Adriano 1997; Shiowatana et al. 2001; Wenzel et al. 2001). These extraction protocols have enabled researchers to determine that arsenic is often associated with oxides and hydroxides in soil (McLaren et al. 2006). The exchange surfaces of silicates and organic matter tend to be negatively charged, and therefore have a greater tendency to retain cations. In soils that have a low pH (where positive charges predominate), conditions may favor the retention of As in exchange positions. In soils affected by pyritic materials or one that are associated with pyritic mining, arsenic may be found in large proportions within the residual fraction, indicating that it is bound in its mineral form (i.e., associated with sulfides; Conesa et al. 2008)

The study of how arsenic is fractioned within soils can provide useful information about its mobility, migration, and potential toxicity. The As fraction retained in a labile form, within the soil matrix (soil and water) will be the most biologically active (bioavailable fraction) and the most mobile (soluble fraction) one. Fig. 1 is an illustration that depicts the equilibrium achieved by As among several soil

phases. From an ecological and toxicological point of view, the fraction that contains the contaminants in the soil matrix is the most important one, and should be used as an indicator, when analyzing soil contaminant risks (Mench et al. 2009). Therefore, the soil's solid phase is less important than the liquid phase or the equilibrium established between the solid and liquid phases (Sauvé 2001). To date, there is neither a clear consensus surrounding the concept of bioavailability, nor is there an exact way of defining it, in the context of As. In plants, the bioavailable As fraction would be *the amount of As a plant takes up from the soil*, although this concept has yet to be measured and cannot be predicted (Fitz and Wenzel 2006). The available and unavailable fractions of contaminants tend to be in equilibrium within the soil, but any change in environmental factors (pH, Eh, climate, biology, hydrology, organic matter, etc.), or alterations in mineral content (e.g., from dissolution-precipitation; oxidation-reduction; formation of complexes-disassociation; adsorption-desorption) can alter the availability of an element (Mench et al. 2009). This dynamic behavior notwithstanding, the analysis of soils by many methods have produced interesting results when estimating a contaminant's potential plant bioavailability. The available fraction has generally been measured by correlating amounts of the element found in the soil vs. amounts found within the plants grown in the soil (Feng et al. 2005; Vázquez et al. 2008a). When this approach is used, some neutral salt extraction methods (Vázquez et al 2008a), or those utilizing organic acid mixtures (Feng et al. 2005) have produced satisfactory results. Moreover, monitoring programs that rely on rhizosphere suction cups have been employed, and these are designed to measure the available fraction of interstitial water that occurs within samples (Clemente et al. 2010). One factor that is insufficiently understood is the rate of exchange that takes place between unavailable and available fractions, although that rate appears to be rather slow (Cattani et al. 2009).

2.2 Factors Determining Arsenic Availability in Soils

2.2.1 The Effect of pH and Eh

In contrast to what happens with other trace elements, a rise in pH often results in mobilization of arsenic in the soil. In general, a rise in soil pH causes a release of anions from within their exchange positions, such that arsenate and arsenite are released (Smith et al. 1999; Fitz and Wenzel 2002; Beesley et al. 2010b; Moreno-Jiménez et al. 2010a). However, several experiments (mainly with mine soils) have shown that high pH values, in the presence of sulfates and carbonates, can produce either a co-

precipitation of arsenic in the subsequently formed oxyhydroxides and sulfates (García et al. 2009), or a precipitate such as calcium arsenate (which is slightly less insoluble than calcium phosphate) (Burriel et al. 1999). For this reason, some soils probably demonstrate their maximum As(V) retention at a pH near 10.5 (Goldberg and Glaubig 1988). In well aerated alkaline soils, the solubility of As is limited by its precipitation as Ca or Fe arsenates (Xie and Naidu 2006). In soils with a high pH, carbonates can play an important role in the retention of arsenate (Zhang and Selim 2008). When the pH drops below 2.5, As(V) becomes completely protonated (Zhang and Selim 2008), rendering it less likely to be retained by soil particles.

As(V) is the predominant form that exists in soils, in which the pH+pe>10; in contrast, As(III) is the dominant form found in soils, in which the pH+pe is less than 6 (Sadiq 1997). Under aerobic conditions, sulfides are easily oxidized, and as a consequence arsenic is released into the environment (Adriano 2001); when soil pH is between 3 and 13, the major species found are $H_2AsO_4^-$ and $HAsO_4^{2-}$ (Smedley and Kinninburgh 2002). In reducing environments, arsenic is found as arsenite the predominant species of which is H_3AsO_3. Poor adsorption of As(III) results from its neutral character in soils (Lakshmipathiraj et al. 2006). Arsenite is more mobile and more toxic than is arsenate. Poor adsorption occurs when the redox potential of the soil is negative (Fitz and Wenzel 2002), and changes in the handling or conditions of soils results in speciation changes of As (Carbonell-Barrachina et al. 2004). Highly reducing conditions can cause As co-precipitation with iron-sulfurs, such as aresenopyrite, or the formation of arsenic sulfides (AsS, As_2S_3). During the oxidation of pyrite, Fe is oxidized from valence II to III, and arsenic is oxidized to arsenate. In contrast, under reducing conditions, Fe and Mn oxides are dissolved, releasing arsenate that is rapidly reduced to arsenite (Gräfe and Sparks 2006).

2.2.2 The Role of Fe, Al and Mn Oxides and Oxyhydroxides

Soils frequently retain important quantities of Fe, Al and Mn oxides and oxyhydroxides. The distribution of these solids in the soil depends on both the pH and Eh of the soils involved. Under reducing conditions, the structure of Fe and Mn hydroxides is broken, and arsenic that was fixed to the interior or to the surface of these compounds is released. The activity of arsenic in the soil solution is controlled by reactions of retention and release along the surfaces of Fe, Mn, and Al oxides and hydroxides (Livesey and Huang 1981; Fitz and Wenzel 2002; de Brouwere et al. 2004),

and soils with a large quantity of iron had a greater retention capacity of both arsenate and arsenite (Manning and Goldberg 1997), arsenite being retained in lower quantities than arsenate (Fitz and Wenzel 2002). As (V) has a high affinity for the surfaces of iron oxides, where it forms inner-sphere complexes**;** however, As (V) can also be retained in external-sphere complexes (Waychaunas et al. 1993; Cheng et al. 2008). The results of several studies have shown that As(III) can be adsorbed and oxidized along the surfaces of some Fe-oxyhydroxides, such as goethite and ferrihydrite, or those of Mn (such as birmesite; Lin and Puls 2000). In other studies, it was demonstrated that the adsorption of As(V) on goethite, magnetite, and hematite is reduced when the pH is raised (Manning et al. 1997; Giménez et al. 2007). Giménez et al. (2007) found that hematite had the largest sorption capacity, followed by goethite and then magnetite. Arsenate has a high affinity for the surfaces of iron oxides, as phosphate; however, arsenate has a lower affinity for aluminum oxides than phosphate (Adriano 2001). Under reducing conditions, when a large portion of the Fe and Mn oxides have been reduced, gibbsite (which is more thermodynamically stable in anaerobic conditions) is able to absorb some of the As released by other oxides (Mello et al. 2006). The adsorption of arsenic onto oxides depends on the duration of the interaction between As and the oxide, the release of arsenic being more difficult as the interaction time increases (Gräfe and Sparks 2006).

The addition of Fe to the soil in several forms immobilizes As. For example, additions of Fe oxides, iron-rich soils (those reddish in color), inorganic Fe salts or industrial byproducts, rich in Fe, together with $CaCO_3$, have all been used to raise the quantity of soil oxides, which, in turn, immobilizes As (Hartley et al. 2004; Hartley and Lepp 2008; Vithanage et al. 2007).

2.2.3 Concentration of P and other Elements in the Soil

The phosphate anion, the major species of P present in soils (Marschner 1995), is an analog to arsenate. The application of P to soil results in a release of retained As (Fitz and Wenzel 2002; Cao et al. 2003). This release results from competition between the retention of both anions. Such ions not only compete non-specifically for anionic exchange positions, but they also compete in complexation reactions or in the retention by oxides. When exchange positions are involved, there are competitor ions that are less efficient than phosphorus in displacing arsenic. Phosphate and arsenate are more effectively retained than are other anions, such as Cl^- and NO_3^-, which are rapidly

mobilized. This could result from the fact that chlorides and nitrates are adsorbed with little specificity along the colloidal surface, whereas phosphates and arsenates are specifically adsorbed in soils components. Roy et al. (1986) were able to show that the retention capacity of As(V) was lowered in the presence of phosphate ions. In a similar study, the presence of anions other than phosphate had no effect on As(V) retention (Livesey and Huang 1981). Woolson (1973) demonstrated how the application of phosphorous fertilizers in soils, contaminated by arsenic, mobilized up to 77% of the total arsenic found in the soil, and increased the availability of arsenic. It has been shown in numerous other studies that the application of P in soils causes an increase in the extractable fraction of As. This increase, however, is not necessarily reflected by a greater absorption of As by plants, because arsenate and phosphate are competitors also for absorption by plants (Esteban et al. 2003).

In addition to phosphate, As interacts with other anions. Increasing ionic strength of a soil solution is therefore one method used to reduce the quantity of As retained, and provoke competition between anions for exchange positions (Gräfe and Sparks 2006). The action of anions, other than phosphate, appears to be significant only in the absence of phosphate. Therefore, Stachowicz et al. (2008) observed that, in the absence of phosphate, carbonates moderately compete with arsenate for exchange positions, but when phosphorus is present, the effects of carbonates were not significant. Alternatively, cations can alter the retention/mobilization of As in soils. Smith et al. (2002) determined that the presence of Ca^{2+} and Na^{+} causes an increase in the retention of As. Similarly, Stachowicz et al. (2008) described how Ca^{2+} and Mg^{2+} can induce the adsorption of phosphate and arsenate in soils.

2.2.4 The Effect of Clay Minerals

In general, the availability of arsenic is greater in sandy than in clay soils (Adriano 2001), although the retention of As in clays is less efficient than with oxides (Gräfe and Sparks 2006). Again, As(III) adsorbs to clay minerals less intensely than does As(V). There are many factors involved in the soil adsorption and desorption processes that affect As mobility. Among these factors is the structure of primary and secondary minerals to which As comes into contact. Another factor is the duration of interaction that exists between the clays of a soil and arsenic; the adsorption of As(V) and As(III) by clay minerals increases with time (Lin and Puls 2000). Zhang and Selim (2008) suggest that isomorphic substitutions of Fe by Al in clays contribute to the

adsorption of As. The anionic character of arsenic suggests that the mechanism of clay adsorption of this metalloid may be similar to that of P, through calcium-bridging mechanisms (Fixen and Grove 1990). Frost and Griffin (1977) reported that montmorillonite can adsorb more As(V) and As(III) than does kaolonite, and the difference is derived from the increased surface charge of montmorollonite. Lin and Puls (2000) found that halosite and chlorite clays had a greater capacity to adsorb As(V) than did other clay minerals, and that kaolonite and ilite/montmorollonite, adsorb As (V) to a moderate degree. Arsenic is initially adsorbed on the clay surface, but with time, it is incorporated into the structure of the mineral. It was demonstrated that Californian soils having a greater percent of clay and appreciable quantities of Fe oxides had a greater As retention capacity (Manning and Goldberg 1997).

2.2.5 Interactions with Organic Matter

Organic matter is of a heterogeneous chemical nature, and constitutes a series of organic compounds of variable molecular weights that are differentially polymerized. This soil fraction is dominated elementally by carbon, oxygen, hydrogen, nitrogen and phosphorus (in this order), and commonly has the following functional groups: carboxyls, carbonyls, alcohols and amines (Stevenson 1982). The level of polymerization of humic compounds and their molecular weights influence their solubility: as these compounds diminish in size, they have a greater proportion of functional groups (organic, fulvic and humic acids), and display higher solubility. If more highly polymerized, humic compounds have fewer functional groups, e.g., humines, and will display lower water solubility. The effect organic matter has on trace elements depends on the qualitative composition of the organic matter. An organic fraction that has a large molecular weight will more effectively retain trace elements, whereas a more soluble and lighter fraction tends to dissolve elements, either by chelating (metals) or by displacing (anions) them. Depending on what the predominant compound in the soil is, either of these effects will be observed in the soil.

How soil organic matter affects arsenic is inconsistent: in some studies, the application of organic matter reduced the mobility of arsenic (Gadepalle et al. 2007), whereas, in others As is released after the application of compost (or there is a higher correlation between soluble carbon and soluble arsenic in soils; Mench et al. 2003; Clemente et al. 2008). Weng et al. (2009) have recently studied how fulvic and humic acids in solution are able to reduce the capacity for arsenate retention in goethite

through electrostatic competition. Therefore, dissolved organic matter can compete with arsenate and arsenite for soil retention positions (Bernal et al. 2009). Alternatively, some humic acids may form humic-clay complexes that have the capacity to retain As (Saada et al. 2003). Therefore, the relationship between soil arsenic and organic matter is complex and depends on multiple factors that include: the ratio of soluble organic carbon present, and the fractions of insoluble and stable humus, and the concentrations of Fe, Al, and Mn present in the organic matter (Gräfe and Sparks 2006). One important consideration, when adding organic amendments to a soil, is that the bioavailable fraction of As may be high (Beesley and Dickinson 2010), despite the fact that the total concentration of As is usually <30 mg kg^{-1} (Adriano 2001).

2.2.6 Other Factors

Large differences in various soil parameters may exist during the year, and hence, the availability of trace elements in soil may also be variable (Vanderlinden et al. 2006). These variations result from changes in soil physical properties (humidity, aeration, porosity, temperature, etc.), chemical changes (pH, appearance of precipitates, E, etc.), and biological changes (microbial activity, vegetation cover, etc.). Depending on the concentration of As present and the humidity regime in the soil, precipitates of insoluble salts (e.g., Fe and Ca arsenates, or co-precipitates with jarosite, gypsum or calcite) can form (de Brouwere et al. 2004; Zhang and Selim 2008; Cheng et al. 2008; Kreidie et al. 2011). Such precipitates are frequent in mining sites, where high concentrations of As exist in the soil; if As concentrations are high and rainfall takes place the risk that As will leach from soil will increase. The duration of the interaction between arsenic contamination and the soil is another factor that must be considered, since the bioavailability of As decreases as it ages in soil (favoring its retention in less available fractions; Lombi et al. 1999).

3 Arsenic in Plants

3.1 Absorption and Transport

Different arsenic species simultaneously exist in soils (e.g., As(III), As(V), MMA, DMA; Takamatsu et al. 1982). Where the inorganic form of As predominate, the arsenic is primarily taken up into plants via root absorption, in a process analogous to how nutrients and other trace elements are absorbed.

3.1.1 Changes to As Mobility

Plant roots use fairly efficient mechanisms to modify the solubility and availability of mineral elements in the soil (Marschner 1995). Hence, a plant has a direct influence over the biogeochemical conditions in the area of the root or rhizosphere (Mengel and Kirkby 2001). For example, organic exudates, organic molecules of low molecular weight, are able to mobilize nutrients that are available only in low quantities in the soil, thus rendering anions (phosphates) and cations (Fe, Cu) more available. Alternatively, mechanisms by which plants immobilize toxic soil elements, as is the case of aluminum, have also been described (Mariano and Keljten 2003). It is also known that plants are capable of altering the pH of the rhizosphere (Marschner 1995), thanks to the release of organic acids that serve as soil solution buffers. Moreover, most plants establish relationships with microorganisms (fungus and bacteria) at the root level that also influence the biogeochemical cycle in the rhizosphere. If bacterial activity in the rhizosphere is particularly high, methylation, reduction, or other forms of bacteria-based biochemical activity is favored (Renella et al. 2007). Little is known about the mechanisms by which the availability of toxic soil elements are altered (Kidd et al. 2009), but it is known that the characteristics of the rhizosphere intrinsically depend on the plant species involved.

Although little information exists on the mechanisms by which plants mobilize or immobilize As (Fitz and Wenzel 2002), the analogy drawn between phosphate and arsenate allows us to establish certain parallels between the rhizospheric dynamics of P and As. Many plant species have an active mechanism for pumping organic acids from roots into the rhizosphere, rendering P more mobile (Smith and Read 1997). The mobilization of P induced by organic acids has been attributed to root-influenced pH changes (Raghothama 1999). These organic acids have a low molecular weight (carboxylic acids such as citric and malic acids), and are able to displace phosphate from positions of retention within the soil; they then act to chelate metals that immobilize P, and form metal-chelate complexes with P (Fitz and Wenzel 2002). The soil solubilization of P and its absorption by plants has additionally been attributed to the secretion of flavonoids from roots (Tomasi et al. 2008). Because arsenate and phosphate are chemically analogous, all of these processes are likely to mobilize As; for example, organic acids are capable of displacing arsenate from exchange positions in soils (Redman et al. 2002; Wenzel 2009). Additionally, plant strategies to attack oxides-

hydroxides of Fe will also alter the surfaces on which As is retained, and this can potentially solubilize As (Fitz and Wenzel 2002).

Mycorrhizae are associations between plant roots and fungi, wherein the fungi are able to colonize roots, either intra- or extra-cellularly (depending on the type of mycorrhiza formed). Hence, mycorrhizae play an important role in the mineral nutrition and absorption of elements by plants (Marschner 1995; Azcón-Aguilar and Barea 1997; Fitz and Wenzel 2002). Mycorrhizal interactions are produced in approximately 80% of angiosperms and in all gymnosperms (Fitz and Wenzel 2006), and in many cases provide plants with increased resistance to biotic and abiotic stresses (Latch 1993; Schutzendubel and Polle 2002). Phosphorus is particularly important: many mycorrhizae play a fundamental role in P nutrition in the plant (Mengel and Kirkby 2001), and act to increase, by 2-3 times, the amount of P accumulated in plants (Fitz and Wenzel 2006). Therefore, the study of mycorrhizae is relevant to an understanding of how As/P interact in plants. As absorption is reduced in the presence of mycorrhizae in several plant species, including *Pteris vittata*, lentils, alfalfa, tomatoes, sunflowers and corn (Trotta et al. 2006; Ahmed et al. 2006; Chen et al. 2007; Liu et al. 2005; Ultra et al. 2007a; Yu et al. 2009). Corn mycorrhizae were able to significantly reduce the amount of arsenate, but not arsenite, absorbed by roots (Yu et al. 2009). In this same experiment, a reduction in arsenate reductase activity also occurred in the root from a fungal infection. Some authors attributed the lower concentrations of As in the plants infected with mycorrhizae to a blockage of absorption (Yu et al. 2009), whereas others attributed it to a dilution effect observed from the increase in overall plant mass (Chen et al. 2007).

3.1.2 Absorption of Arsenic

Root absorption of elements first occurs by diffusion from the soil solution within the root apoplast, followed by the symplast, effecting penetration to the interior of plant cells (Menguel and Kirkby 2001). At the cellular membrane, ions enter via transport proteins that are often specific for one or several elements of similar chemical characteristics. Aquaporins and phosphate transporters both are involved in the transmembrane transport and absorption of arsenic.

Aquaporins are water channels; however, other non-charged molecules, like arsenious acid, also enter through them. Various authors have addressed aquaporins in relation to the absorption of As(III) (Isayenkov and Maathuis 2008; Ma et al. 2008).

Interestingly, it has been experimentally determined that As(III) and Si both share the same methods of entrance into and transport in rice (Ma et al. 2008). Hence, there is a group of aquaporins (NIPs) that play a fundamental role in the absorption of non charged molecules, such as glycerol, ammonia, and the boric, arsenious and silic acids (Zhao et al. 2009).

Physiological studies in plants have illustrated the important role that phosphate carriers play in the absorption of arsenate, and the interaction that occurs between both P and As(V) at the root uptake level (Meharg and Macnair 1992; Esteban et al. 2003). The phosphate/arsenate mechanism of absorption involves the co-transport of the anion with protons, in a stoichiometry of $2H^+$ for each anion (Zhao et al. 2009).

Organic forms of As are absorbed less effectively than are inorganic As forms (Marín et al. 1992; Raab et al. 2007; Zhao et al. 2009), and Si transporters may also be involved (Li et al. 2009a).

3.1.3 Accumulation and Transport

Once inside the cell, arsenate is reduced to arsenite, which consumes reduced glutathione: $AsO_4^{-3} + 2\ GSH \rightarrow AsO_3^{-3} + GSSG$, a reaction that is catalyzed by arsenate reductase (Verbruggen et al. 2009). It has therefore been shown that the majority of arsenic that exists in plant tissue is present as arsenite (Tripanthi et al. 2007), regardless of what was in the solution at the time of plant growth. Arsenite has a high affinity for SH groups and tends to be complexed and stored in vacuoles, although it can also be transported via the xylem to other plant tissues. Movement through the xylem is controlled by the flow of the transpiration stream, but is also influenced by membrane transport proteins. Recently, two transporters (Lsi1 and Lsi2) were described between plant endodermis and exodermis, and these mediate the entrance of arsenite into the xylem or its efflux to the external medium (Zhao et al. 2009). These Lsi transporters are principally involved in Si nutrition (Ma et al. 2008).

The transport of As, in most plant species, is generally not very effective and, hence, As tends to remain in roots. An exception exists for those plants that are unusually effective at accumulating As in aerial plant parts. Accumulation of arsenite in the vacuole may be one reason for why As transport into the xylem is reduced (Zhao et al. 2009). Xylem transport of As has been intensely studied over the past years, giving interesting, although somewhat contradictory results. In many plant species, the reduction of As(V) in roots appears to be a key factor that results in blocking the xylem

427 transport of As. Since phosphate is an anion that is completely mobile within plants, one
428 would expect As(V) to act similarly (given the analogy between both anions), but this is
429 not the case in any plant species. The influence of reduced As to block its transport was
430 tested in plants (*Arabidopsis thaliana*) mutated to silence arsenate reductase. These
431 plants demonstrated a ratio of $[As]_{Aerial\ tissue}$:$[As]_{Root}$ that was 25 times greater than the
432 wild type (Dhankher et al. 2006). These authors suggest that this change results from a
433 greater proportion of As(V) being available for transport through the xylem in roots,
434 presumably through the same pathways that handles phosphate.
435 The majority of As that does not accumulate in aerial tissue exists as As(III)
436 (Pickering et al. 2000; Dhankher et al. 2002; Castillo-Michel et al. 2007; Xu et al.
437 2007). The reducing process in roots may constitute a physiological mechanism by
438 which plants limit the flow of As into aerial tissues, thus protecting them from the
439 effects of this metalloid. Additionally, the majority of As in the root is found as
440 complexes (Vázquez et al. 2005), and there is a negative correlation between the percent
441 of As complexed by –SH groups in roots vs. the amounts translocated to aerial tissue
442 (Huang et al. 2008). In this respect, Raab et al. (2005) proposed that complexed As is
443 not transported through the xylem. In hyper-accumulator plants, some authors have
444 utilized synchrotron and liquid chromatography, coupled to ICP-MS techniques, to
445 determine how As is transported from roots to aerial tissues as As(V), and how As is
446 later stored in leaves as As(III) (Zhao et al. 2003; Pickering et al. 2006; Hokura et al.
447 2006; Tripathi et al. 2007). Duan et al. (2005), however, reported that arsenate reductase
448 has greater activity in the roots of *P. vittata*, postulating that As transported through the
449 xylem in this fern is primarily in the form of arsenite. Notwithstanding, in both plants
450 that accumulate arsenic and in those that do not, it appears that once inside the
451 cytoplasm, As is stored in vacuoles, thus avoiding interference with normal cellular
452 function (Verbruggen et al. 2009). Other trace elements are generally stored in tissues
453 that are less metabolically active, like epidermic (Vázquez et al. 1992), or senescing
454 tissues (Ernst et al. 2000).
455 Baker (1981) established a classification that grouped plants into three
456 categories, based on how much element they accumulated in shoots. The three classes
457 are called excluders, indicators and accumulators, and these terms will be used
458 henceforth below. Some species are able to hyper-accumulate As: *Pteris vittata* (up to
459 22,000 mg As kg^{-1}), *Jasione montana* (6640), *Calluna vulgaris* (4130), *Agrostis tenuis*

and *stolonifera* (10000), *Pityrogramma calomelanos*, *Mimosa púdica*, *Melastoma malabratrhicum* (8350) (Wang and Mulligan 2006). Except for these particular plants, most plants accumulate arsenic in their roots. This, however, does not exclude the fact that many plants are adapted to grow in soils that have high As concentrations, without accumulating it. Among such plants are the tolerant excluder ecotypes. The concentration of arsenic in non-accumulator plants rarely exceeds 2 mg As kg^{-1} in aerial parts (Horswell and Speir 2006). From an agricultural perspective, areas flooded with waters rich in As may pose a problem, because these crops may contain sufficient As levels to render them dangerous for human consumption (Bhattacharya et al. 2007). In Fig. 2, links between physiological traits in plants and As-phytoaccumulation strategies are presented.

3.2 Arsenic Toxicity in Plants

When present within plant cells, As has various negative physiological effects, many of which have recently been the subject of intense study. Arsenic has no known biological function, although positive effects have been described at low concentrations of arsenate. The reason behind these positive effects has yet to be determined. Although these effects may be attributed to As itself, they may also result from an increase in the absorption of P when As concentrations remain below toxic thresholds (Carbonell-Barrachina et al. 1998). Arsenite is generally more toxic than arsenate, partially because of its greater solubility and mobility. The sensing and signaling of an excess of As in plants and the complex biochemical changes it induces are still unknown, although they have been extensively studied in the recent years (Verbruggen et al. 2009). When the toxicity of trace elements is studied in plants, herbaceous plants and/or crops are generally the target species used. Woody species are uncommonly used in As plant toxicity testing, despite the fact that they are favored when attempts are made to revegetate degraded soils.

The toxicity of organoarsenics has been scarcely studied in plants; however, they appear to block mersistematic tissues, and also affect protein synthesis (Horswell and Speir 2006).

3.2.1 Visual Effects

Impairment of plant development is one of the observed symptoms from arsenic toxicity. Arsenate and arsenite both reduce the growth of plant species, such as *Holcus lanatus*, *Lupinus albus* and *Triticum aestivum*, when grown under hydroponic

493 conditions (Hartley-Whitaker et al. 2001; Vázquez et al. 2005; Geng et al. 2006).
494 Among the symptoms induced by As exposure in plants are reduced root elongation,
495 loss of root branching, chlorosis in leaves, and shrinking or necrosis in aerial plant parts
496 (Carbonell-Barrachina et al. 1998).

497 **3.2.2 Oxidative Stress**

498 Elements that have several oxidation states often serve as good reaction catalysts
499 in which oxidation-reduction is involved (As, Cu, Hg, etc.). In aqueous solutions of near
500 neutral pH, such as in the cytoplasm, radicals can produce H_2O_2, which later produce
501 hydroxyl radical. All reactive oxygen species (ROS) can directly damage biomolecules
502 and cause peroxidation of membrane lipids. Arsenic can also induce oxidative stress in
503 plant cells, a fact reflected as an increase in the concentration of malondialdehyde
504 (MDA) in vegetable tissues; MDA is a metabolic product of the peroxidation of lipids
505 in biological membranes. The stimulation of oxidative stress, associated with the
506 accumulation of arsenic in plants, has been shown to occur in both cultivated plants, and
507 in wild bushes (Hartley-Whitaker et al. 2001; Mascher et al. 2002; Moreno-Jiménez et
508 al. 2008, 2009a). Arsenic also modifies the gene expression involved in cellular
509 homeostasis for redox perturbation (Requejo and Tena 2005) and activates some
510 antioxidant enzymes (Srivastava et al. 2005).

511 **3.2.3 Nutritional Disorders**

512 Plant mineral nutrition is affected in a non-specific manner by many toxic
513 elements. Effects result either from an alteration in transport processes of the cellular
514 membrane, or effects on transpiration. The most notable effect produced by As results
515 from the similarities that exist between arsenate and phosphate. Addition of arsenate
516 causes a decrease in the levels of P in plants, because the entry of both ions is mediated
517 through the same membrane transporter (Meharg and Macnair 1992; Vázquez et al.
518 2005). Among other changes to nutritional patterns caused by the presence of As in the
519 environment in which plants are grown, is a decrease in the concentrations of Mn, Fe,
520 Cu, N, Zn and Mg (Mascher et al. 2002; Vázquez et al. 2008b).

521 **3.2.4 Photosynthetic Inhibition**

522 Arsenic causes chlorosis in plant leaves because of the induced decrease in
523 chlorophyll levels (Mascher et al. 2002). There may be other reasons for As-induced
524 chlorosis (such as iron deficiency), but the primary cause is directly attributed to the
525 inhibition of pigment synthesis (Jain and Gadre 1997). The effects produced result

from limited availability of δ-aminolevulinic acid (a precursor to chlorophyll) and from alterations in proteins rich in thiol groups.

3.2.5 Metabolic and Genetic Alterations

Theoretically, arsenate can substitute for phosphate in some metabolic processes. Within the cytoplasm, arsenate competes with phosphate in phosphorylation reactions, such as the synthesis of ATP. Herein, ADP-As is formed, altering the energetics of the cell cycle because of its instability (Verbruggen et al. 2009). Arsenite demonstrates a high affinity for –SH groups of biomolecules (enzymes and proteins), inhibiting their function (Meharg and Hartley-Whitaker 2002). As(III) and (AsV) are mutagenic compounds that can alter the genome (Lin et al. 2008). For this reason, the presence of repair biomolecules in the chromatin may be able to increase a plant's tolerance to arsenic (Verbruggen et al. 2009).

3.3 Mechanisms of Arsenic Resistance in Plants

There is a difference between plant resistance and plant tolerance to toxic elements. Resistance to toxic elements is generally defined by a plant's capacity to support an excess of a toxic element present in the environment. By comparison, tolerance is the exceptional capacity of a plant to survive in a soil that is toxic to other plants, demonstrated by the interaction between genotype-environment (Hall 2002). Ernst et al. (2008) have established another classification, based on the tolerance/sensitivity a plant has to trace elements: (i) hypotolerant, sensitive, or hypersensitive plants are those with genetically modified phenotypes that are extremely vulnerable to one or many metals or metalloids; (ii) basal tolerant --would be equivalent to resistance, meaning genetic resistance of one species (also called non-metallic populations or populations with constitutive tolerance); (iii) hypertolerants are populations that show a low sensitivity to one or more elements due to adaptive mechanisms (also called metalophytes). Plants resist the effects of toxicity by using several biological mechanisms, all of which are known to be detoxification mechanisms. There is still insufficient research information available to explain what cellular mechanisms plants use to detect the presence of As. Moreover, what signals are triggered after As is detected and detoxification mechanisms initiated is also not known, other than those cellular signals derived from the tissue damage produced (Verbruggen et al. 2009).

3.3.1 Mycorrhization

559 Mycorrhizae are able to modify tolerance patterns in host plants (Sharples et al.
560 2000; González-Chávez et al. 2002; Leung et al. 2006). In regard to the effects that
561 mycorrhizae cause in their hosts, plants can be categorized as follows: (i) plants that
562 respond positively to mycorrhization, with increases in biomass and improvements in
563 mineral nutrition (AM-r plants), and (ii) plants in which mycorrhization has no effect
564 (n-AM-r plants). Smith et al. (2010) discussed how mycorrhization effectively
565 alleviated the toxic effects of As on AM-r plants, whereas literature references on those
566 same effects on n-AM-r plants are variable. In wild rye**,** for example, some plants
567 infected with mycorrhizae (n-AM-r) showed no improved tolerance to As (Knudson et
568 al. 2003), while in other cases, many plants infected with mycorrhizae did show
569 tolerance improvement. When tolerance was improved in plants infected with
570 mycorrhizae, there were several different reasons for the improvement. Xu et al. (2009),
571 for example, attributed improvement to greater absorption of P and a reduced
572 concentration of As in *Medicago truncatula* plants, while Yu et al. (2009) explained this
573 same effect as an inhibition in the absorption and speciation of As in plants infected
574 with mycorrhizae.

575 **3.3.2 Immobilization Within the Rhizosphere**

576 It has been shown that, in flooded soils, iron plaques are formed in areas
577 surrounding the roots. These plaques are able to retain high concentrations of As, and
578 act as the first filter at the root level. This mechanism is especially efficient in rice
579 paddies or where plants grown in liquid media (Hansel et al. 2002; Liu et al. 2004,
580 2006). In the rhizosphere of aerated soils, redox reactions favor the formation of iron
581 plaques that retain As in the areas directly surrounding roots. Fe, retained in the
582 apoplastic area surrounding roots of plants grown in well aerated soils, could act in a
583 similar manner as that of flooded soils (Doucleff and Terry 2002).

584 **3.3.3 Exclusion**

585 Exclusion reduces the entrance of As via changes in the mechanisms of root
586 absorption. Tolerant populations of the plant *Holcus lanatus* lack the high affinity
587 uptake system for phosphate absorption (Meharg and Macnair 1992). For this reason,
588 these plants had reduced absorption of both phosphate and arsenate, and were better
589 adapted to environments having high concentrations of As (Meharg and Hartley-
590 Whitaker 2002). *Arabidopsis thaliana* plants, in the presence of As(V), slowed gene
591 expression associated with phosphate absorption; simultaneously, the plant induced

592 transcriptional gene expression that was stimulated by As, suggesting that there are
593 distinct transcriptional pathways that regulate the deficiency of P and that the responses
594 to As are interconnected (Catarecha et al. 2007).

595 **3.3.4 Active Efflux**

596 Arsenic can be pumped out of plant cells (Xu et al. 2007), mainly in the form of
597 arsenite. Although this mechanism has yet to be clearly established, the status of the
598 information that exists on the subject was recently summarized by Zhao et al. (2009).
599 Active efflux of arsenite has been observed to occur in various plant species grown
600 under hydroponic conditions: wheat, barley, corn, tomato, *Holcus lanatus* and
601 *Arabidospsis thaliana*. Physiological evidence indicates that the expulsion process is
602 active and depends on a proton gradient created by cellular metabolism. It has yet to be
603 demonstrated that increased expulsion correlates directly with improved plant tolerance
604 within a given plant population; however, this relationship has been demonstrated in
605 microorganisms (Bhattacharjee and Rosen 2007). Studies performed in soil have
606 provided evidence that arsenite accumulates in areas directly surrounding the roots of
607 sunflower and corn (Ultra et al. 2007a,b; Vetterlein et al. 2007). It is estimated that up
608 to 50-80% of the As absorbed by roots may be secreted via active efflux in non-
609 accumulating plants (Verbruggen et al. 2009). In contrast, it has been demonstrated that
610 hyper-accumulating plants do not show As efflux to the environment via roots (Zhao et
611 al. 2009).

612 **3.3.5 Complexation**

613 Plants take advantage of the high affinity arsenite has for –SH groups to
614 deactivate the toxic effects of As. Therefore, there are many ligands in the cytosol for
615 which trace elements have a high affinity. In certain plant species, As(III) complexes
616 exist that have different biomolecules rich in thiol groups such as glutathione and
617 phytochelatines (Meharg and Hartley-Whitaker 2002). For example, it has been shown
618 that the presence of some trace elements, such as Ag, As, Cd, Cu, Hg, Se or Pb in
619 plants, provoke the synthesis of phytochelatines. Phytochelatines (PCs) are small
620 peptides, rich in cysteine, that have the general structure (y-Glu-Cys)n-Gly, where n-2-
621 11. PCs are bound to trace elements via thiolate bonds that are synthesized from
622 glutathione (GSH) and catalyzed by the enzyme PCsynthase. PCs are able to effectively
623 complex inorganic arsenic that accumulates in plants, and GS_3-As(III) complexes have
624 been identified to exist in certain plants (Pickering et al. 2000). Many plant species have

responded to the presence of As in the environment, such as lupin, *Holcus lanatus*, sunflower or *Silene vulgaris* (Sneller et al. 2000; Hartley-Whitaker et al. 2001; Vázquez et al. 2005; Raab et al. 2005; Aldrich et al. 2007; Moreno-Jiménez et al. 2008, 2009a). *Arabidopsis* plants that have a greater capacity to synthesize PCs were more tolerant than were the wild types (Dhanker et al. 2002); moreover, resistant clones of *H. lanatus* showed a greater production of PCs in response to As than sensitive plants (Hartley-Whitaker et al. 2001). The foregoing points indicate that PCs, and probably GSH play a role in the detoxification of As. However, this mechanism requires great metabolic effort and, in some instances, greater tolerance has not been correlated to greater levels of phytochelatines or thiols. Therefore, in hyper-accumulating plants, this detoxification method appears to be limited in comparison to the high levels of As that accumulate (Zhao et al. 2003). And, there is some evidence that the proportion of As complexed by SH in the root is negatively correlated with the translocation of As to the aerial portion of the plant (Huang et al. 2008).

3.3.6 Compartmentalization

Once arsenite is complexed, it is most probably stored in the vacuoles of root cells, which reduces its mobility in the cytoplasm and its translocation through the xylem. It is believed, however, that vacuolar compartmentalization, and thereby, detoxification of As, is important in all plant organs (Pickering et al. 2006). Once inside the vacuole, where the pH is approximately 8, the rupture of the complex could take place, and the ligand could then be used to complex more arsenite. Although there is no experimental data to show that the entrance of PC-As or GSH-As through the tonoplast takes place under in vivo conditions (Tripanthi et al. 2007), it is known to occur under *in vitro* conditions (Dhanker et al. 2006). The entrance of such arsenite forms that are complexed to thiol groups is most likely facilitated via ABC-(ATP binding cassette superfamily) type transporters (Verbruggen et al. 2009).

Some authors have suggested that As is retained in cell walls (Doucleff and Terry 2002; Vázquez et al. 2007), although this has not clearly been established. Cell wall retention has been demonstrated to be an effective detoxification mechanism for other trace elements, such as Cd and Hg (Zornoza et al. 2002; Moreno-Jiménez et al. 2007).

4 Practical Applications for Mitigating Arsenic's Effects

4.1 Phytoremediation

Phytoremediation is the use of plants to remediate organic or inorganic contaminates of soils and water (Salt et al. 1995). Chaney et al. (1997) defined soil phytoremediation as the use of plants, amendments, or agricultural techniques to eliminate, retain or reduce the toxicity of soil contaminants. From the onset, phytoremediation was seen as a competitive technology for recuperating soils. It has promised and has produced positive results, which makes it attractive to both the commercial and scientific communities (Peuke and Rennenberg 2005). The benefits that phytoremediation has shown over competitive techniques (physico-chemical site-cleaning methods) are as follows: (i) it is inexpensive, (ii) it is less invasive, and (iii) it is well received both environmentally and socially. Phytoremediation has been successfully used to clean arsenic-contaminated soils and water.

4.1.1 Phytoextraction

Phytoextraction is the technique plants use to accumulate significant quantities of a contaminant in their tissues, and these plants can later be harvested or collected for appropriate disposal or management (McGrath and Zhao 2003). This technology has been studied and applied to events connected with Cd, Zn, Pb, Ni and As contamination. Except for Ni, which has economic value (the technique with Ni clean-up is called phyto-mining; Chaney et al. 2007), the other forms of contaminated plant biomass pose a disposal problem (Robinson et al. 2006). In some cases, phytoextraction has been assisted by mobilizing agents, although the experiments have shown that one must be careful to control leaching, because there is high risk that the contaminant could disperse into water sources (Nowack et al. 2006). McGrath and Zhao (2003) estimated that, if one plant produced 10 t of biomass ha^{-1}, it would need to accumulate a contaminant (soil/plant concentration ratio) by 20 fold to reduce it to 50% of the original soil level, after 10 plant cycles. Phytoextraction of As (Fig. 3) has been tested using in-container studies that utilize the hyper-accumulating plant species *Pteris vittata*, although other hyper-accumulating plants are available (Meharg 2005). A recent and cogent review was published that was specific to As accumulation in *P. vittata (*Xie et al. 2009).

P. vittata has root-level transporters that have high affinity and capacity for As absorption (Poyton et al. 2004). In addition, this species shows a limited root complexing of As (Zhao et al. 2003), and elevated concentrations of As in sap (Su et al.

691 2008). Together, these characteristics trigger an extraordinary level of translocation of
692 As towards the aerial portion of the plant (Tu and Ma 2002). Although As is usually not
693 that bioavailable from soils, the rhizosphere of *P. vitatta* appears to be uniquely capable
694 of mobilizing As, even from soil fractions that are barely available (Fitz et al. 2003). A
695 reduction in redox potential, and an increase in the amount of organic carbon released in
696 the rhizosphere of ferns may be what enhances As uptake by the plant. Moreover,
697 following phytoextraction with this plant, the available As fraction was reduced.
698 Unfortunately, the distribution and ecological niche of *Pteris vittata* is restricted, which
699 confines its application to specific zones. In addition, there is the problem of having to
700 dispose of the harvested (contaminated) plant tissue. Recently, two reviews have been
701 authored, in which the necessity of improving the genetics of phytoextracting plants
702 through molecular plant biology was described (Tripathi et al. 2007; Zhu and Rosen
703 2009).

704 It has recently been determined that the As fraction retained in labile form is
705 recharged at a slow rate, which impedes phytoextraction (Cattani et al. 2009). With
706 respect to assisted phytoextraction of As, results have revealed that As absorption by
707 plants increased after application of phosphate (Tassi et al. 2004) or biodegradable
708 chelating agents such as HIDS (Hydroxyiminodisuccinic Acid) and EDDS
709 (Ethylenediamine-N,N'-disuccinic Acid) (Azizur-Rahman et al. 2009). In this case, it is
710 necessary to carefully evaluate the consequence of diffuse contamination, when As
711 becomes more soil mobile (Peñalosa et al. 2007).

712 **4.1.2 Phytostabilization**

713 Phytostabilization is the mixed use of plants and agricultural practices to reduce
714 mobilization and transfer of contaminants (Chaney et al. 1997). When plants are present
715 in the soil, contaminants are stabilized, making them less susceptible to erosion and
716 wind dispersion. Plants accumulate contaminants in the root, which further impedes
717 their transfer and mobility. When this occurs, auto-sustainable plant species that have a
718 prolonged life cycle and are adapted to such environmental conditions are especially
719 interesting. The concept of natural attenuation consists of an ecosystem's and soil's
720 capacity to auto-regulate and react slowly to chemical attacks, which can potentially
721 reduce the risks associated with the presence of the contaminating element (Adriano et
722 al. 2004). Assisted natural attenuation refers to the use of techniques such as replanting
723 or the application of amendments to accelerate the process of natural attenuation

(Madejón et al. 2006). For As, it has been demonstrated that both natural attenuation as well as phytostabilization could be useful field techniques (Vázquez et al. 2006; Madejón and Lepp 2007; Domínguez et al. 2008; Moreno-Jiménez et al. 2010a, 2011b).

The authors of some studies, however, have described plants that have little influence on the available As fraction, particularly when As exists at high soil concentrations (King et al. 2008). Phytostabilization is aided when organic or inorganic amendments are used; these materials improve soil properties by retaining metalloids or preventing their solubilization (de la Fuente et al. 2009). Kumpiene et al. (2008) reviewed different amendments that could immobilize As in soils. They concluded that the amendments could be both useful and effective in managing arsenic contamination. Among useful inorganic amendments are those rich in iron (red sludge, rolling mill scale, etc.), fly ash, clays or liming materials (Kumpiene et al. 2008; de la Fuente et al. 2010), whereas organic amendments capable of immobilizing As are those that have high stability of the humus (de la Fuente et al. 2009). In either case, it is necessary to monitor contaminants over time to assure that the phytostabilization process has been a success (Vangronsveld et al. 2009).

Recently, phyto-attenuation has been described as the process by which a plant extracts the most available fraction of an element from the soil, which can later be harvested in such a way that it reduces the risks associated with inorganic soil contamination (Meers et al. 2010). Phytostabilization of arsenic in roots has been confirmed as a useful remediation technique, because there is low risk of As being remobilized, even when the roots themselves are mineralized (Vázquez et al. 2008c; Moreno-Jiménez et al. 2009b).

4.1.3 Phytofiltration

The presence of As in water presents one of the greatest of environmental risks to human health; As presents both a direct risk through consumption, as well as through indirect risk from contaminated irrigation waters. Phytofiltration is a technique that uses plants/roots to decontaminate water (Raskin et al. 1997). Aquatic, semi-aquatic, and terrestrial plants, and plant biomass (Haque et al. 2007) have all been used to eliminate or reduce arsenic contaminated water. Ideally, plants used in phytofiltration must be effective in capturing the contaminant from water, and must also grow rapidly. Plants that are not adapted to grow in anoxic conditions or those that are unable to grow rapidly in such conditions, may require the water in which they grow to be artificially

aerated. Blastofiltration is the use of plant seedlings instead of plants. Seedling use increases the surface-area ratio of contact/volume of water, allowing better contaminant absorption in plant material (Raskin et al. 1997). Anawar et al. (2008) recently reviewed the use of phytofiltration in water, concluding that this method could be an effective alternative. Two strategies to deal with As contamination by this method have been studied: (i) employing plants that need a support structure and (ii) using species of plants that float on water. The first strategy relies on *P. vittata* to eliminate As from solutions by growing the plants under hydroponic conditions in contaminated water (Malik et al. 2009). Plants can purify solutions that retain up to 200 $\mu g\ L^{-1}$ of As, and can lower the concentration present to less than 10 $\mu g\ L^{-1}$, in only 24 hours (Huang et al. 2004). In other experiments, design parameters were optimized to achieve maximal As uptake by stabilizing the pH below 5.2 (Tu and Ma 2003). In the second strategy, plants of the genus *Lemna* and the macrophyte *Spirodela polyrhiza* were able to reduce the concentration of As in contaminated waters (Azizur-Rahman et al. 2007; Sasmaz and Obek 2009).

4.2 Reducing Arsenic Transfer to Edible Plant Tissues

The main sources of arsenic exposure to humans are through consuming contaminated water (Hurtado-Jiménez and Gardea-Torresdey 2006) or food (Meharg et al. 2008). In some countries, there is a high risk of exposure to arsenic from eating contaminated foods (Meharg et al. 2009).

4.2.1 Presence of Arsenic in Rice

Rice is the principal source of human nutrition in much of the world. It is commonly grown in naturally or artificially flooded soils. When rice is grown under conditions of poor aeration, arsenic is primarily found as As(III), which is highly mobile. In this form, As is easily absorbed by rice plants, whose capacity to accumulate As is greater than most other crops, such as wheat or barley (Williams et al. 2007). As is stored in rice tissues at increasing concentrations in the following parts: external iron plaque>root>straw>husk>grain (Liu et al. 2006). Therefore, formation of an external iron plaque inhibits, in part, the accumulation of As in the rice plant (Liu et al. 2006).

Regardless of order, the concentrations of As that appear in edible portions of rice are very high, especially considering the quantities of rice eaten by humans. Meharg et al. (2009) have shown that eating rice is the main source of As exposure in many countries, and increases risks associated with chronic arsenic exposure. Liao et al.

790 (2010) described how consuming contaminated rice is associated with skin disease in
791 children. The average concentrations of As in rice are between 0.05 and 0.95 mg kg^{-1},
792 although some samples exceed 1 mg As kg^{-1} (Williams et al. 2005). It has been
793 estimated that the quantity of arsenic consumed per person, per day, in some countries,
794 may be up to 0.9 mg As day^{-1} (Butcher 2009). According to the WHO, this intake
795 amount approaches the maximum tolerable limit for daily ingestion of As (Williams et
796 al. 2005).

797 Experiments in recent years have shown that the development of agricultural and
798 genetic techniques may help control or reduce such risks (Tripathi et al. 2007; Zhao et
799 al. 2009). One approach to risk reduction involves selecting varieties of rice that
800 accumulate less As in their edible parts (Williams et al. 2005). Alternatively, the same
801 end may be achieved by using genetic techniques (Tripathi et al. 2007). Lemont,
802 Azucena and Te-qing are rice cultivars that apparently accumulate less As in their seeds
803 (Norton et al. 2009). Genetic modification studies are also being conducted to evaluate
804 methods by which As accumulation in edible tissues can be reduced, or As
805 volatilization increased from edible tissues (Tripathi et al. 2007).

806 Work to mitigate As levels in crops through changes in agricultural practices
807 have also been attempted (Fig.3). Using As-contaminated water for crop irrigation has
808 increased As uptake from soils into plants for decades (Williams et al. 2006; Khan et al.
809 2009). Using clean irrigation water, or purifying the water before use in irrigation would
810 obviously reduce food levels of arsenic. Improving aeration of soils would help
811 immobilize any As present, and would reduce its plant availability (Xu et al. 2008).
812 As(III) is absorbed through plant roots via water channels that also are involved in the
813 absorption of boron and silicon (Ma et al. 2008). Competition between these elements
814 and arsenite may therefore exist, which would explain why rice fertilized with Si had
815 lower concentrations of As (Guo et al. 2009; Li et al. 2009b). Finally, consumers can
816 reduce their As exposure risk by boiling rice in abundant water (Raab et al. 2009), or by
817 substituting rice with other grains, such as wheat or corn, if possible (Signes-Pastor et
818 al. 2009).

819 **4.2.2 Selecting Crops for Low As Levels**

820 Reducing the oral intake of As in humans by reducing the amounts consumed in
821 crops would greatly improve food safety. Some crops accumulate higher levels of As
822 than others do. Since As is generally retained in plant roots (Moreno-Jiménez et al.

2008; Zhang et al. 2009), tubers and edible roots may accumulate As, and it would be most wise to closely evaluate these where they are heavily consumed. Potatoes plants (Moyano et al. 2009) and other tubers grown in As -contaminated soils, such as radishes (Warren et al. 2003), carrots, garlic and onions (Huang et al. 2006; Zhao et al. 2009), have shown significant levels of As. In contrast to crops showing high As levels, de la Fuente et al. (2010) reported only low or moderate levels of As in potatoes, carrots and sugar beets.

Other plant organs are also subjected to As accumulation. Huang et al. (2006) described the following pattern of As concentrations in the edible parts of the following edible crops: celery>mustard>spinach>lettuce>taro>bokchoi >cowpea>cauliflower >eggplant. De la Fuente et al. (2010) reported the following pattern in several crops: red cabbage~curly endive>barley~wheat~sugar beet~leek>cabbage~green pepper. Zhao et al. (2009) found As concentrations to be high in lettuce, eggplant, and green onions, whereas, Gulz et al. (2005) found As concentrations in sunflower and rape to be greater than those of corn. Warren et al. (2003) reported concentrations of As to be greater in lettuce and broccoli than in spinach, but above all, these authors found the greatest concentrations of As to occur in radishes. The percentage of inorganic As in edible crops was reported to be high, although the concentration found posed no serious risk for human consumption (de la Fuente et al. 2010). Gulz et al. (2005) advised against using sunflower and rape seeds to produce cooking oil, because As levels in these crops surpassed the maximum levels permitted in Switzerland of 0.2 mg As kg^{-1}.

4.2.3 Using Soil Amendments and Mycorrhizae

Applying inorganic amendments can affect the mode of uptake and level of available As in soils. Particularly effective are amendments rich in Fe, because iron oxides effectively retain As (Mench et al. 2003; Warren et al. 2003; Hartley and Lepp 2008; de la Fuente et al. 2010). Fertilization with P also alters the absorption of As, because phosphate displaces arsenate in the soil and mobilizes it. Gunes et al. (2008) reported an increase in As accumulation when P was applied; however, the application of phosphorus-based fertilizers reduced the As plant levels (Khattak et al. 1991; Pigna et al. 2009).

Raising the soil pH increases As plant bioavailability (Smith et al. 1999; Fitz and Wenzel 2002), although some studies indicate that application of $CaCO_3$ to acidic soils reduced As availability (Simón et al. 2005).

856 The application of organic amendments increases the soil mobility of, and
857 therefore, As plant uptake (Mench et al. 2003; Hartley and Lepp 2008; Renella et al.
858 2007; Clemente et al. 2010), but some studies show that available As is stabilized with
859 the application of organic matter to soil (Gadepalle et al. 2007). The fact that organic
860 matter is both stable and is highly polymerized may help to explain such contradictory
861 results. Combining Fe-enriched amendments with organic materials should be a future
862 focus of research, since these amendments provide a good environment for plant
863 growth, and reduce or
864 prevent the transfer of harmful As or other metal concentrations to edible plant
865 tissues.
866 Ninety percent of higher plants interact with mycorrhizae (González-Chávez et
867 al. 2002; Leung et al. 2006; Chen et al. 2007) and mycorrhizae appear to affect the soil
868 behavior of As. Existing data suggest that plants infected with mycorrhizae have a
869 greater ratio of P/As in their tissues, and this bestows greater As tolerance on them
870 (Smith et al. 2010). In some studies, the reduction of As in plants that were infected by
871 mycorrhizae was attributed to two effects: (i) a slower rate of root absorption of As (Yu
872 et al. 2009), and (ii) a dilution effect from accentuated plant growth (Smith et al. 2010).
873 Further research is needed to find improved plant-microorganism combinations that
874 minimize As accumulation in plant tissues destined for human consumption.
875 **4.3 Alternatives: Using Contaminated Crops for Non-food Purposes**
876 An alternative to consuming As-contaminated food, is to use As-contaminated
877 biomass for non-food purposes (Vangronsveld et al. 2009). Such biomass could be used
878 in energy production or as primary material inputs for industrial products (Thewys
879 2008; Dickinson et al. 2009; Pandey et al. 2009; Mench et al. 2010). In either case, the
880 use of phytotechnologies is viable for managing contaminated soils and as means to
881 return them to economic profitability (Thewys and Koppens 2008). The examples in
882 which such alternative uses were actually put into practice are few, but, when used, the
883 results have proven satisfactory; notwithstanding, improvements to these techniques are
884 required (Thewys and Koppens 2008). Several experiments were conducted in
885 contaminated soils using plant species such as *Salix* spp., corn, sunflower, tobacco,
886 poplar, *Brassica* ssp., *Pteris vittata*, etc. (Vangronsveld et al. 2009). Energy from such
887 biomass could be in the form of biogas generation, direct incineration, pyrolysis,
888 biomass gasification, fermentation into biofuels, etc. (Mench et al. 2010). Degraded

889 sites can be managed to produce energy and at the same time reduce the environmental
890 risk associated with arsenic. For this purpose, contaminated soils may be used to amend
891 wastes (compost, biochar, or iron oxides), thus recycling the materials. Both renewable
892 energy production and human waste recycling are key factors for the future global
893 environmental agenda (Dickinson et al. 2009). Crops grown in contaminated soils could
894 also be used to produce other non-food goods. Examples are: cosmetics, industrial
895 products, essential oils, paper, cardboard, wood, plant fibers, etc. (Mench et al. 2010).
896 Regardless of the end use to which contaminated plant biomass is put, avoiding
897 unacceptable environmental impact is crucial, particularly when arsenic or other
898 contaminants are present in plant biomass.
899
900 **5 Summary**
901 Arsenic is a natural trace element found in the environment. In some cases and
902 places, human activities have increased the soil concentration of As to levels that exceed
903 hazard thresholds. Amongst the main contributing sources of As contamination of soil
904 and water are the following: geologic origin, pyritic mining, agriculture and coal
905 burning. Arsenic speciation in soils occurs and is relatively complex. Soils contain both
906 organic and inorganic arsenic species. Inorganic As species include arsenite and
907 arsenate, which are the most abundant forms found in the environment. The majority of
908 As in aerated soils exists as $H_2AsO_4^-$ (acid soils) or $HAsO_4^{2-}$ (neutral and basic).
909 However, H_3AsO_3 is the predominant species in anaerobic soils, where arsenic
910 availability is higher and As(III) is more weakly retained in the soil matrix than is
911 As(V). The availability of As in soils is usually driven by multiple factors. Among these
912 factors is the presence of Fe-oxides, and/or phosphorus, (co)precipitation in salts, pH,
913 organic matter, clay content, rainfall amount, etc. The available and most labile As
914 fraction can potentially be taken up by plant roots, although the concentration of this
915 fraction is usually low.
916 Arsenic has no known biological function in plants. Once inside root cells,
917 As(V) is quickly reduced to As(III), and, in many plant species, becomes complexed.
918 Phosphorus nutrition influences As(V) uptake and toxicity in plants, whilst silicon has
919 similar influences on As(III). Plants cope with As contamination in their tissues by
920 possessing detoxification mechanisms. Such mechanisms include complexation, and
921 compartmentalization. However, once these mechanisms are saturated, symptoms of

phytotoxicity appear. Phytotoxic effects commonly observed from As exposure includes growth inhibition, chlorophyll degradation, nutrient depletion and oxidative stress. Plants vary in their ability to accumulate and tolerate As (from tolerant hyperaccumulators to sensitive excluders), and some plants are useful for soil reclamation and in sustainable agriculture.

The status of current scientific knowledge allows us to manage As contamination in the soil-plant system and to mitigate arsenic's effects. Phytoremediation is an emerging technology suitable for reclaiming As-contaminated soils and waters. Phytoextraction has been used to clean As-contaminated soils, although its applicability has not yet reached maturity. Phytostabilization has been employed to reduce environmental risk by confining As as an inert form in soils, and has shown success in both laboratory experiments and in field trials. Phytofiltration has been used to treat As-enriched waters. Such treatment removes As when it is accumulated in plants grown in or on water. In agricultural food production, appropriate soil management and plant variety/species selection can minimize As-associated human diseases and the transfer of As within the food chain. Selecting suitable plants for use on As contaminated soils may also enhance alternative land use, such as for energy or raw material production.

Keywords: arsenate, arsenite, phytoremediation, phytotoxicity, crops, environmental risk

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References

Adriano DC (2001) Trace elements in terrestrial environments. Biogeochemistry, bioavailability and risks of metals. Springer-Verlag, New York.

954 Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS (2004) Role of assisted natural
955 remediation in environmental cleanup. Geoderma 122:121-142.

956 Ahmed FRS, Killham K, Alexander I (2006) Influences of arbuscular mycorrhizal
957 fungus *Glomus mosseae* on growth and nutrition of lentil irrigated with arsenic
958 contaminated water. Plant Soil 283:33–41.

959 Aldrich MV, Peralta-Videa JR, Parsons JG, Gardea-Torresdey JL (2007) Examination
960 of arsenic(III) and (V) uptake by the desert plant species mesquite (*Prosopis* spp.)
961 using X-ray absorption spectroscopy. Sci Tot Environ 379:249-255.

962 Allen HE (2001) Terrestrial ecosystem: an overview. In: Allen HE, (ed) Bioavailability
963 of Metals in Terrestrial Ecosystems: Importance of Partitioning for Availability to
964 Invertebrates, Microbes, and Plants. SETAC Press, pp. 1-6, Pensacola, Florida.

965 Anawar HM, Garcia-Sanchez A, Regina IS (2008) Evaluation of various chemical
966 extraction methods to estimate. Plant-available arsenic in mine soils. Chemosphere
967 70:1459–1467.

968 Atker K, Naidu R (2006) Arsenic speciation in the environment. In: Naidu R. et al. (eds)
969 Managing Arsenic in the Environment. From Soils to Human Health. CSIRO Pub.,
970 Collingwood, Australia, pp. 61-74.

971 Azcón-Aguilar C, Barea JM (1997) Applying mycorrhiza biotechnology to horticulture:
972 significance and potentials. Sci Hortic 68:1-24.

973 Azizur Rahman M, Hasegawa H, Mahfuzur Rahman M, Arifur Rahman M, Miah MAM
974 (2007) Accumulation of arsenic in tissues of rice plant (*Oryza sativa* L.) and its
975 distribution in fractions of rice grain. Chemosphere 69:942-948.

976 Azizur-Rahman M, Hasegawa H, Kadohashi K, Maki T, Ueda K (2009)
977 Hydroxyaminodisuccinic acid (HIDS): A novel biodegradable chlating ligand for the
978 increase of iron bioavailability and arsenic phytoextraction. Chemosphere 77:207-
979 213.

980 Baker AJM (1981) Accumulators and excluders: strategies in the response of plants to
981 heavy metals. J Plant Nutr 25:369-386.

982 Barán EJ (1995) Química Bioinorgánica. McGraw-Hill, Madrid.

983 Beesley L, Dickinson N (2010) Carbon and trace element mobility in an urban soil
984 amended with green waste compost. J Soils Sedim 10:215-222.

985 Beesley L, Moreno-Jiménez E, Clemente R, Lepp N, Dickinson N (2010a) Mobility of
986 arsenic, cadmium and zinc in a multi-element contaminated soil profile assessed by
987 in-situ soil pore water sampling, column leaching and sequential extraction. Environ
988 Pollut 158:155-160.

989 Beesley L, Moreno-Jiménez E, Gómez-Eyles JL (2010b) Effects of biochar and
990 greenwaste compost amendments on mobility, bioavailability and toxicity of
991 inorganic and organic contaminants in a multi-element polluted soil. Environ Pollut
992 158:2282-2287.

993 Bernal MP, Clemente R, Walker DJ (2009) Interaction of heavy metals with soil
994 organic matter in relation to phytoremediation. In: Navarro-Aviño JP Ed.,
995 Phytoremediation: The Green Salvation of the World. Research Signpost, Kerala,
996 India, pp. 109-129.

997 Bhattacharjee H, Rosen BP (2007) Arsenic metabolism in prokaryotic and eukaryotic
998 microbes. In: Nies DH, Silver S Eds., Molecular microbiology of heavy metals.
999 Springer-Verlag, Berlin, pp. 371–406.

1000 Bhattacharya P, Welch AH, Stollenwerk KG, McLaughlin MJ, Bundschuh J, Panaullah
1001 G (2007) Arsenic in the environment: biology and chemistry. Sci Tot Environ
1002 379:109-120.

1003 Bissen M, Frimmel FH (2003) Arsenic- a review. Part I; Ocurrence, toxicity, speciation,
1004 mobility. Acta Hydrochim Hydrobiol 31:9-18.

1005 Burriel F, Lucena F, Arribas S, Hernández J (1999) Química Analítica Cualitativa.
1006 Paraninfo, Madrid. Apéndice VI.

1007 Butcher DJ (2009) Phytoremediation of arsenic: fundamentals studies, practical
1008 applications, and future prospects. Appl Spectrom Rev 44:534-551.

1009 Cao X, Ma LQ, Shiralipour A (2003) Effects of compost and phosphate amendments on
1010 arsenic mobility in soils and arsenic uptake by the hyperaccumulator *Pteris vittata* L.
1011 Environ Pollut 126:157–167.

1012 Carbonell-Barrachina AA, Aarabi MA, DeLaune RD, Gambrell RP, Patrick WH Jr
1013 (1998) The influence of arsenic chemical form and concentration on *Spartina patens*
1014 and *Spartina alterniflora* growth and tissue arsenic concentration. Plant Soil 198:33–
1015 43.

1016 Carbonell-Barrachina AA, Rocamora A, García C (2004) Arsenic and zinc
1017 biogeochemistry in pyrite mine wate from the Aznalcóllar environmental disaster.
1018 Geoderma 122:195-203.

1019 Castillo-Michel H, Parsons JG, Peralta-Videa JR, Martínez-Martínez A, Dokken KM,
1020 Gardea-Torresdey JL (2007) Use of X-ray absorption spectroscopy and biochemical
1021 techniques to characterize arsenic uptake and reduction in pea (*Pisum sativum*)
1022 plants. Plant Physiol Biochem. 45:457-463.

1023 Catarecha P, Segura MD, Franco-Zorrilla JM, Garcia-Ponce B, Lanza M, Solano R,
1024 Paz-Ares J, Leyva A (2007) A mutant of the Arabidopsis phosphate transporter
1025 PHT1;1 displays enhanced arsenic accumulation. Plant Cell 19:1123-1133.

1026 Cattani I, Capri E, Boccelli R, del Re AAM (2009) Assessment of arsenic availability to
1027 roots in contaminated Tuscany soils by diffusive gradients in thin films(DGT)
1028 method and uptake by *Pteris vittata* and *Agrostis castellana*. Eur J Soil Sci 60:539-
1029 548.

1030 Caussy D (2003) Case studies of the impact of understanding bioavailability: arsenic.
1031 Ecotoxicol Environ Saf 56:164-173.

1032 Chaney RL, Malik KM, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997)
1033 Phytoremediation of soil metals. Current Opin. Biotechol. 8:279-284.

1034 Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007)
1035 Improved understanding of hyperaccumulation yields commercial phytoextraction
1036 and phytomining technologies. J Environ Qual 36:1429-1443.

1037 Chang P, Kim JY, Kim KW (2005) Concentrations of arsenic and heavy metals in
1038 vegetation at two abandoned mine tailings in South Korea. Environ Geochem Health
1039 27:109-119.

1040 Chen B, Xiao D, Zhu X, Smith G, Xie FA, Smith S E (2007) The arbuscular
1041 mycorrhizal fungus *Glomus mosseae* gives contradictory effects on phosphorus and
1042 arsenic acquisition by *Medicago sativa* Linn. Sci Tot Environ 379:226–234.

1043 Cheng H, Hu Y, Luo J, Zhao J (2008) Geochemical processes controlling fate and
1044 transport of arsenic in acid mine drainage (AMD) and natural systems. J Hazar Mater
1045 165:13-26.

1046 Clemente R, Dickinson NM, Lepp NW (2008) Mobility of metals and metalloids in a
1047 multi-element contaminated soil 20 years after cessation of the pollution source
1048 activity. Environ Pollut 155:254-261.

1049 Clemente R, Hartley W, Riby P, Dickinson NM, Lepp NW (2010) Trace element
1050 mobility in a contaminated soil two years after field-amendment with a greenwaste
1051 compost mulch. Environ Pollut 158:1644-1651.

1052 Conesa HM, Robinson BH, Schulin B, Nowack B (2008) Metal extractability in acidic
1053 and neutral mine tailings from the Cartagena-La Unión Mining District (SE Spain).
1054 Appl Geochem. 23:1232–1240

1055 De Brouwere K, Smolders E, Merckx R (2004) Soil properties affecting solid-liquid
1056 distribution of As(V) in soils. Eur J Soil Sci 55:165-173.

de la Fuente C, Alburquerque JA, Clemente R, Martínez-Alcalá I, Bernal MP (2009) Estrategias de Recuperación de Suelos Enriquecidos con Arsénico en la Provincia de Segovia. Imprenta Comercial Segoviana, Segovia, España.

de la Fuente C, Clemente R, Alburquerque JA, Vélez D, Bernal MP (2010) Implications of the use of As-rich groundwater for agricultural purposes and the effects of soil amendments on As solubility. Environ Sci Technol 44:9463-9469.

DEFRA (2002) Soil Guideline Values for Arsenic Contamination. Environmental Agency, Bristol, UK.

Dhankher OP, Li Y, Rosen BP, Shi J, Salt D, Senecoff JF, Sashti NA, Meagher RB (2002) Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthetase expression. Nat Biotechnol 20:1140-1145.

Dhankher OP, Rosen BP, McKinney EC, Meagher RB (2006) Hyperaccumulation of arsenic in the shoots of *Arabidopsis* silenced for arsenate reductase (ACR2). Proc Nat Acad Sci 103:5413–5418.

Dickinson NM, Baker AJM, Doronilla A, Laidlaw S, Reeves RD (2009) Phytoremediation of inorganics: realism and synergies. Int. J. Phytoremediat. 11:97-114.

Domínguez MT, Marañón T, Murillo JM, Schulin R, Robinson BH (2008) Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain: A large-scale phytomanagement case study. Environ Pollut 152:150-159.

Doucleff M, Terry N (2002) Pumping out the arsenic. Nat Biotechnol 20:1094–1095.

Duan GL, Zhu YG, Tong YP, Cai C, Kneer R (2005) Characterization of arsenate reductase in the extract of roots and fronds of Chinese brake fern, an arsenic hyperaccumulator. Plant Physiol 138:461–469.

Ernst WHO, Nelissen HJM, Ten Bookum WM (2000) Combination toxicology of metal enriched soils: physiological responses of a Zn- and Cd-resistant ecotype of *Silene vulgaris* on polymetallic soils. Environ Exp Bot 43:55–71.

Ernst WHO, Krauss GJ, Verkleij JA, Wesenberg D (2008) Interaction of heavy metals with the sulphur metabolism in angiosperms from an ecological point of view. Plant Cell Environ 31:123-143.

Esteban E, Carpena RO, Meharg AA (2003) High-affinity phosphate/arsenate transport in white lupin (*Lupinus albus*) is relatively insensitive to phosphate status. New Phytol 158:165-173.

1091 Feng MH, Shan XQ, Zhang SZ, Wen B (2005) Comparison of a rhizosphere-based
1092 method with other one-step extraction methods for assessing the bioavailability of
1093 soil metals to wheat. Chemosphere 59:939–949.

1094 Fixen PE, Grove JH (1990) Testing soils for phosphorus. In: Soil Testing and Plant
1095 Analysis, 3rd Ed., Westerman RL Ed., Soil Science Society of America, Inc.
1096 Madison, Wisconsin, USA. pp. 141-180.

1097 Fitz WJ, Wenzel WW (2002) Arsenic transformations in the soil-rhizophere-plant
1098 system: fundamentals and potential application to phytoremediation. J Biotechnol
1099 99:259-278.

1100 Fitz WJ, Wenzel WW, Zhang H, Nurmi J, Stipek K, Fischerova Z, Schweiger P,
1101 Köllensperger G, Ma LQ, Stingeder G (2003) Rhizosphere characteristics of the
1102 arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval
1103 efficiency. Environ Sci Technol 37:5008-5014.

1104 Fitz WJ, Wenzel WW (2006) Sequestration of arsenic by plants. In: Naidu R et al. (eds)
1105 Managing Arsenic in the Environment. From Soils to Human Health. CSIRO Pub.,
1106 Collingwood, Australia, pp. 209-222.

1107 Frost RR, Griffin RA (1977) Effect of pH on adsorption of arsenic and selenium from
1108 landfill leachate by clay minerals. Soil Sci Soc A J 41:53-57.

1109 Gadepalle VP, Ouki SK, Van Herwijnen R, Hutchings T (2007) Immobilization of
1110 heavy metals in soil using natural and waste materials for vegetation establishment
1111 on contaminated sites. Soil Sedim Contam 16:233-251.

1112 García I, Diez M, Martín F, Simón M, Dorronsoro C (2009) Mobility of arsenic and
1113 heavy metals in a Sandy-loam textured and carbonated soil. Pedosphere 19:166-175.

1114 Geng CN, Zhu YG, Tong YP, Smith SE, Smith FA (2006) Arsenate (As) uptake by and
1115 distribution in two cultivars of winter wheat (*Triticum aestivum* L.). Chemosphere
1116 62:608–615.

1117 Giménez J, Martínez M, de Pablo J, Rovira M, Duro L (2007) Arsenic sorption onto
1118 natural hematite, magnetite, and goethite. J Hazar Mater 141:575-580.

1119 Goldberg S, Glaubig RA (1988) Anion sorption on a calcareous, montmorillonitic soil –
1120 arsenic. Soil Sci Soc A J 52:1154-1157.

1121 González-Chávez C, Harris PJ, Dood J, Meharg AA (2002) Arbuscular mycorrhizal
1122 fungi confer enhanced arsenic resistance on *Holcus lanatus*. New Phytol 155:163-
1123 171.

1124 Gräfe M, Sparks DL (2006) Solid phase speciation of arsenic. In: Naidu R, et al. Eds.
1125 Managing Arsenic in the Environment. From Soils to Human Health. CSIRO Pub.,
1126 Collingwood, Australia, pp. 75-92.

1127 Gulz PA, Gupta SK, Schulin R (2005) Arsenic accumulation of common plants from
1128 contaminated soils. Plant Soil 272:337-347.

1129 Gunes A, Pilbeam DJ, Inal A (2008) Effect of arsenic–phosphorus interaction on
1130 arsenic-induced oxidative stress in chickpea plants. Plant Soil 314:211-220.

1131 Guo W, Zhang J, Teng M, Li LH (2009) Arsenic uptake is suppressed in a rice mutant
1132 defective in silicon uptake. J Plant Nutr Soil Sci 172:867-874.

1133 Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp
1134 Bot 53:1–11.

1135 Haque MN, Morrison GM, Perrusquíz G, Gutiérrez M, Aguilera AF, Cano-Aguilera I,
1136 Gardea Torresdey JL (2007) Characteristics of arsenic adsorption to sorghum
1137 biomass. J Hazar Mater 145:30-35.

1138 Hansel CM, La Force MJ, Fendorf S, Sutton S (2002) Spatial and temporal association
1139 of As and Fe species on aquatic plant roots. Environ Sci Technol 36:1988–1994.

1140 Hartley-Whitaker J, Ainsworth G, Meharg AA (2001) Copper- and arsenate-induced
1141 oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. Plant Cell
1142 Environ 24:713–722.

1143 Hartley W, Edwards R, Lepp NW (2004) Arsenic and heavy metal mobility in iron
1144 oxide-amended contaminated soils as evaluated by short- and long-term leaching
1145 tests. Environ Pollut 131:495–504.

1146 Hartley W, Lepp NW (2008) Remediation of arsenic contaminated soils by iron-oxide
1147 application, evaluated in terms of plant productivity, arsenic and phytotoxic metal
1148 uptake. Sci Tot Environ 390:35-44.

1149 Horswell J, Speir T (2006) Arsenic phytotoxicity. In: Naidu R, et al. Eds. Managing
1150 Arsenic in the Environment. From Soils to Human Health. CSIRO Pub.,
1151 Collingwood, Australia, pp. 183-208.

1152 Hokura A, Omuma R, Tereda Y, Kitajima N, Abe T, Saito H, Yoshida S, Nakai I (2006)
1153 Arsenic distribution and speciation in an arsenic hyperaccumulator fern by X-ray
1154 spectroscopy utilizing a synchrotron radiation source. J Anal Atom Spectrom 21:321-
1155 328.

1156 Huang JW, Poynton CY, Kochian LV, Elless MP (2004) Phytofiltration of arsenic from
1157 drinking water using arsenic-hyperaccumulating ferns. Environ Sci Technol 38:3412-
1158 3417.

1159 Huang R, Gao S, Wang W, Staunton S, Wang G (2006) Soil arsenic availability and the
1160 transfer of soil arsenic to crops in suburban areas in Fujian Province, southeast
1161 China. Sci Tot Environ 368:531-541.

1162 Huang ZC, Chen TB, Lei M, Liu YR, Hu TD (2008) Difference of toxicity and
1163 accumulation of methylated and inorganic arsenic in arsenic-hyperaccumulating and
1164 -hypertolerant plants. Environ Sci Technol 42:5106–5111.

1165 Hurtado-Jiménez R, Gardea-Torredey JL (2006) Arsenic in drinking water in the Los
1166 Altos de Jalisco region of Mexico. Revista Panamericana de Salud Pública 20:236-
1167 246.

1168 Isayenkov SV, Maathuis FJM (2008) The *Arabidopsis thaliana* aquaglyceroporin
1169 AtNIP7;1 is a pathway for arsenite uptake. FEBS Let 582:1625–1628.

1170 Jain M, Gadre RP (1997) Effect of As on chlorophyll and protein contents and enzymic
1171 activities in greening maize tissues. Water Air Soil Pollut 93:109–115.

1172 Kahn MA, Islam MR, Panaullah GM, Duxbury JM, Jahiruddin M, Loeppert RH (2009)
1173 Fate of irrigation-water arsenic in rice soils of Bangladesh. Plant Soil 322:263-277.

1174 Khattak RA, Page AL, Parker DR, Bakhtar D (1991) Accumulation and interactions of
1175 arsenic, selenium, molybdenum and phosphorus in alfalfa. J Environ Qual 20:165-
1176 168.

1177 Kidd P, Barceló J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S, Clemente R,
1178 Monterroso C (2009) Trace element behaviour at the root-soil interface: implications
1179 on phytoremediation. Environ Exp Bot 67:243-259.

1180 King DJ, Doronila AI, Feenstra C, Baker AJM, Woodrow IE (2008) Phytostabilisation
1181 of arsenical gold mine tailings using four Eucalyptus species: Growth, arsenic uptake
1182 and availability after five years. Sci Tot Environ 406:35-42.

1183 Knudson JA, Meikle T, DeLuca TH (2003) Role of mycorrhizal fungi and phosphorus
1184 in the arsenic tolerance of the basin wildrye. J Environ Qual 32:200-2006.

1185 Kreidie N, Armiento G, Cibin G, Cinque G, Crovato C, Nardi E, Pacifico R, Cremisini
1186 C, Mottana A (2011) An integrated geochemical and mineralogical approach for the
1187 evaluation of arsenic mobility in mining soils. J Soil Sedim 11:35-52.

1188 Kumpiene J, Lagerkvist A, Maurice C (2008) Stabilization of As, Cr, Cu, Pb and Zn in
1189 soil using amendments-A review. Waste Manag 28:215-225.

1190 Lakshmipathiraj BRV, Narasimhan S, Prabhakar S (2006) Absorption of arsenate on
1191 synthetic goethite from aqueous solutions. J Hazar Mater 136:281-287.

1192 Latch GCM (1993) Physiological interactions of endophytic fungi and their hists. Biotic
1193 stress tolerance imparted to grasses by endophytes. Agric Ecosys Environ 44:143-
1194 156.

1195 Lepp NW (1981) Effect of heavy metals pollution on plants. Vol. 1. Effects of trace
1196 metal on plant function. Applied Science Publishers, London & New Jersey.

1197 Leung HM, Ye ZH, Wong MH (2006) Interactions of mycorrhizal fungi with *Pteris*
1198 *vittata* (As hyperaccumulator) in As-contaminated soils. Environ Pollut 139:1–8.

1199 Li RY, Ago Y, Liu WJ, Mitani N, Feldmann J, McGrath SP, Ma JF, Zhao FJ (2009a)
1200 The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. Plant Physiol
1201 150:2071-2080.

1202 Li RY, Stroud JL, Ma JF, McGrath SP, Zhao FJ (2009b) Mitigation of arsenic
1203 accumulation in rice with water management and silicon fertilization. Environ Sci
1204 Technol 43:3778-3783.

1205 Liao CM, Li TM, Hsieh NH, Chen WY (2010) Assessing the arsenic-contaminated rice
1206 (*Oryza sativa*) associated children skin lesions. J Hazar Mater 176:239-251.

1207 Lin Z, Puls RW (2000) Adsorption, desorption and oxidation of arsenic affected by clay
1208 minerals and aging processes. Environ Geol 39:753-759.

1209 Lin A, Zhang X, Zhu YG, Zhao FJ (2008) Arsenate-induced toxicity: effects on
1210 antioxidative enzymes and DNA damage in *Vicia faba*. Environ Toxicol Chem
1211 27:413-419.

1212 Liu WJ, Zhu YG, Hu Y, Williams PN, Gault AG, Meharg AA, Charnock JM, Smith FA
1213 (2006) Arsenic sequestration in iron plaque, its accumulation and speciation in
1214 mature rice plants (*Oryza sativa* L.). Environ Sci Technol 40:5730–5736.

1215 Liu WJ, Zhu YG, Smith FA, Smith SE (2004) Do phosphorus nutrition and iron plaque
1216 alter arsenate (As) uptake by rice seedlings in hydroponic culture? New Phytol
1217 162:481–488.

1218 Liu Y, Zhu YG, Chen BD, Christie P, Li XL (2005) Yield and arsenate uptake of
1219 arbuscular mycorrhizal tomato colonized by *Glomus mosseae* BEG167 in As spiked
1220 soil under glasshouse conditions. Environ Int 31:867–873.

1221 Livesey NT, Huang PM (1981) Adsorption of arsenate by soils and its relation to
1222 selected properties and anions. Soil Sci 131:88-94.

1223 Lombi E, Wenzel WW, Sletten RS (1999) Arsenic adsorption by soils and iron-coated
1224 sand: kinetics and reversibility. J Plant Nutr Soil Sci 162:451-456.

1225 Ma JF, Yamaji N, Mitani N, Xu XY, Su YH, McGrath SP, Zhao FJ (2008) Transporters
1226 of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Nat Acad
1227 Sci 105:9931-9935.

1228 Madejón E, Perez de Mora A, Felipe E, Burgos P, Cabrera F (2006) Soil amendments
1229 reduce trace element solubility in a contaminated soil and allow regrowth of natural
1230 vegetation. Environ Pollut 139:40-52.

1231 Madejón P, Lepp NW (2007) Arsenic in soils and plants of woodland regenerated on an
1232 arsenic-contaminated substrate: A sustainable natural remediation? Sci Tot Environ
1233 379:256-262.

1234 Malik AH, Khan ZM, Mehmood Q, Nasreen S, Bhatti ZA (2009) Perspectives of low
1235 cost arsenic remediation of drinking water in Pakistan and other countries. J Hazar
1236 Mater 168:1-12.

1237 Mandal BK, Suzuki KT (2002) Arsenic round the world: a review. Talanta 58:201-235.

1238 Manning BA, Goldberg S (1997) Arsenic (III) and arsenic (V) adsorption on three
1239 California soils. Soil Sci 162:886-895.

1240 Marschner H (1995) Mineral Nutrition of Higher Plants. Academic Press, San Diego.

1241 Marin AR, Masscheleyn PH, Patrick WH (1992) The influence of chemical form and
1242 concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil
1243 139:175-183.

1244 Martín F, Díez M, García I, Simón M, Dorronsoro C, Iriarte A, Aguilar J (2007)
1245 Weathering of primary minerals and mobility of major elements in soils affected by
1246 an accidental spill of pyrite tailing. Sci Tot Environ 378:49-52.

1247 Mascher R, Lippmann B, Holzinger S, Bergmann H (2002) Arsenate toxicity: effects on
1248 oxidative stress response molecules and enzymes in red clover plants. Plant Sci
1249 163:961-969.

1250 Mariano ED, Keljten WG (2003) Evaluating the root of citrate exudation as mechanism
1251 of aluminium resistance in maize. Plant Soil 256:469-479.

1252 Matschullat J (2000) Arsenic in the geosphere – a review. Sci Tot Environ 249:297-312.

1253 McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from
1254 contaminated soils, Current Opin Biotechnol 14:277-282.

1255 McLaren RG, Megharaj M, Naidu R (2006) Fate of arsenic in the soil environment. In:
1256 Naidu R et al. Eds. Managing Arsenic in the Environment. From Soil to Human
1257 Health. CSIRO Publishing, Australia. pp. 157-182.

1258 Meers E, Van Slyken S, Adriaensen K, Ruttens A, Vangronsveld J, Du Laing G,
1259 Thewys T, Tack FMG (2010) The use of bio-energy crops (*Zea mays*) for
1260 'phytoattenuation' of heavy metals on moderately contaminated soils: A field
1261 experiment. Chemosphere 78:35-41.

1262 Meharg AA, Macnair MR (1992) Suppression of the high affinity phosphate uptake
1263 system: a mechanism of arsenate tolerance in *Holcus lanatus* L. J Exp Bot 43:519–
1264 524.

1265 Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic
1266 resistant and nonresistant plant species. New Phytol 154:29–43.

1267 Meharg AA (2005) Mechanisms of plant resistance to metal and metalloid ions and
1268 potential biotechnological applications. Plant Soil 274:163–174.

1269 Meharg AA, Lombi E, Williams PN, Scheckel KG, Feldman J, Raab A, Zhu YG, Islam
1270 R (2008) Speciation and localization of arsenic in white and brown rice grains.
1271 Environ Sci Techno. 42:1051-1057.

1272 Meharg AA, Williams PN, Adomako E, Lawgali YL, Campell RCJ, Sun G, Zhu YG,
1273 Feldman J, Raab A, Zhao FJ, Islam R, Hossain S,. Yanai J (2009) Geographical
1274 variation in total and inorganic arsenic content of polished (white) rice. Environ Sci
1275 Technol 43:1612-1617.

1276 Mello JWV, Roy WR, Talbott JL, Stucki JW (2006) Mineralogy and Arsenic
1277 mobilization in arsenic-rich Brazilian soils and sediments. J Soil Sedim 6:9–19.

1278 Mench M, Bussiere S, Vangronsveld J, Manceau A (2003) Progress in remediation and
1279 revegetation of the barren Jales gold mine spoil after in-situ treatments. Plant Soil
1280 249:187–202.

1281 Mench M, Schwitzguébel JP, Schroeder P, Bert V, Gawronski S, Gupta S (2009)
1282 Assessment of successful experiments and limitations of phytotechnologies:
1283 contaminant uptake, detoxification, and sequestration, and consequences to food
1284 safety. Environ Sci Pollut Res 16:876-900.

1285 Mench M, Lepp N, Bert V, Schwitzguébel JP, Gawronski SW, Schöder P,
1286 Vangronsveld J (2010) Successes and limitations of phytotechnologies at field scale:
1287 outcomes, assessment and outlook from COST action 859. J Soil Sedim 10:1039-
1288 1070.

1289 Mengel K, Kirkby EA (2001) Principles of Plant Nutrition. Kluwer Ac. Publ.
1290 Dordretch, The Netherlands.

1291 Mestrot A, Feldmann J, Krupp EM, Hossain MS, Roman-Ross G, Meharg AA (2011)
1292 Field fluxes and speciation of arsines emanating from soils. Environ Sci Technol
1293 45:1798-1804.

Moreno-Jiménez E, Peñalosa JM, Esteban E, Carpena RO (2007) Mercury accumulation and resistance to mercury stress in *Rumex induratus* and *Marrubium vulgare* grown on perlite. J Plant Nutr Soil Sci 170:485–494.

Moreno-Jiménez E, Peñalosa JM, Carpena-Ruiz RO, Esteban E (2008) Comparison of arsenic resistance in Mediterranean woody shrubs used in restoration activities. Chemosphere 71:466-473.

Moreno-Jiménez E, Esteban E, Carpena-Ruiz RO, Peñalosa JM (2009a) Induced phytotoxicity in mediterranean shrubs *P. lentiscus* and *T. gallica* after arsenic or mercury accumulation. Ecotoxicol Environ Saf 72:1781-1789.

Moreno-Jiménez E, Peñalosa JM, Esteban E, Bernal MP (2009b). Feasibility of arsenic phytostabilisation using Mediterranean shrubs: impact of root mineralization on As availability in soils. J Environ Monitor 11:1375-1380.

Moreno-Jiménez E, Manzano R, Esteban E, Peñalosa JM (2010a) The fate of arsenic in soils adjacent to an old-mine site (Bustarviejo, Spain): mobility and transfer to native flora. J Soil Sedim 10:301-312.

Moreno-Jiménez, E., Esteban, E., Fresno, T, López de Egea, C., and Peñalosa, J.M. (2010b) Hydroponics as a valid tool to assess arsenic availability in mine soils. Chemosphere 79:513-517.

Moreno-Jiménez E, Beesley L, Lepp NW, Dickinson NM, Hartley W, Clemente R (2011a) Field sampling of soil pore water to evaluate trace element mobility and associated environmental risk. Environ Pollut *in press*, doi:10.1016/j.envpol.2011.04.004.

Moreno-Jiménez E, Vázquez S, Carpena-Ruiz RO, Esteban E, Peñalosa JM (2011b) Using Mediterranean shrubs for the phytoremediation of a soil impacted by pyritic wastes in Southern Spain: A field experiment. J Environ Manag 92:1584-1590.

Moyano A, García-Sánchez A, Mayoraga P, Anawar HM, Álvarez-Ayuso E (2009) Impact of irrigation with arsenic-rich groundwater on soils and crops. J Environ Monitor 11:498-502.

Norton GJ, Duan GL, Dasgupta T, Islam MR, Lei M, Zhu YG, Deacon CM, Moran AC, Islam S, Zhao FJ, Stroud JL, McGrath SP, Feldman J, Price AH, Meharg AA (2009) Environmental and genetic control of arsenic accumulation and speciation in rice grain: comparing a range of common cultivars grown in contaminated sites across Bangladesh, China, and India. Environ Sci Technol 43:8381-8386.

Nowack B, Schulin R, Robinson BH (2006) Critical assessment of chelatant-enhanced metal phytoextraction. Environ Sci Technol 40:5225-5232.

1329 Ongley LK, Sherman L, Armienta A, Concilio A, Ferguson Salina C (2007) Arsenic in
1330 the soils of Zimapán, México. Environ Pollut 145:793-799.

1331 Onken BM, Adriano DC (1997) Arsenic availability in soil with time under saturated
1332 and subsaturated conditions. Soil Sci Soc A J 61:746-752.

1333 Padey VC, Abhilash PC, Singh N (2009) The Indian perspective of utilizing fly ash in
1334 phytoremediation, phytomanagement and biomass production. J Environ Manag
1335 90:2943-2958.

1336 Peñalosa JM, Carpena RO, Vázquez S, Agha R, Granado A, Sarro MJ, Esteban E
1337 (2007) Chelate-assisted phytoextraction of heavy metals in a soil contaminated with
1338 a pyritic sludge. Sci Tot Environ 378:199-204.

1339 Peuke AD, Rennenberg H (2005) Phytoremediation. EMBO Rep 6:497-501.

1340 Pickering IJ, Prince RC, George MJ, Smith RD, George GN, Salt DE (2000) Reduction
1341 and coordination of arsenic in Indian mustard. Plant Physiol 122:1171–1178.

1342 Pickering IJ, Gumaelius L, Harris HH, Prince RC, Hirsch G, Banks JA, Salt DE, George
1343 GN (2006) Localizing the biochemical transformations of arsenate in a
1344 hyperaccumulating fern. Environ Sci Technol 40:5010–5014

1345 Pigna M, Cozzolino V, Violante A, Meharg AA (2009) Influence of phosphate on the
1346 arsenic uptake by wheat (*Triticum durum* L.) irrigated with arsenic solutions at three
1347 different concentrations. Water Air Soil Pollut 197:371-380.

1348 Poynton CY, Huang JW, Blaylock MJ, Kochian LV, Elless MP (2004) Mechanisms of
1349 arsenic hyperaccumulation in *Pteris* species: root As influx and translocation. Planta
1350 219:1080-1088.

1351 Raab A, Schat H, Meharg AA, Feldmann J (2005) Uptake, translocation and
1352 transformation of arsenate and arsenite in sunflower (*Helianthus annuus*): formation
1353 of arsenic–phytochelatin complexes during exposure to high arsenic concentrations.
1354 New Phytol 168:551–558.

1355 Raab A, Williams PN, Meharg A, Feldmann J (2007) Uptake and translocation of
1356 inorganic and methylated arsenic species by plants. Environ Chem 4:197–203.

1357 Raab A, Baskaran C, Feldman J, Meharg AA (2009) Cooking rice in a high water to
1358 rice ratio reduces inorganic arsenic content. J Environ Monitor 11:41-44.

1359 Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove
1360 pollutants from the environment. Current Opin Biotechnol 8:221-226.

1361 Raghothama KG (1999) Phosphate adquisition. Ann Rev Plant Physiol Plant Mol Biol
1362 50:665-693.

1363 Redman AD, Macalady DL, Ahmann D (2002) Natural organic matter affects arsenic
1364 speciation and sorption onto hematite. Environ Sci Technol 36:2889-2896.

1365 Requejo R, Tena M (2005) Proteome analysis of maize roots reveals that oxidative
1366 stress is a main contributing factor to plant arsenic toxicity. Phytochem 66 :1519-
1367 1528.

1368 Renella G, Landi L, Valori F, Nannipieri P (2007) Microbial and hydrolase activity
1369 after release of low molecular weight organic compounds by a model root surface in
1370 a clayey and sandy soil. Appl Soil Ecol 36:124-129.

1371 Robinson B, Schulin R, Nowack B, Roulier S, Menon M, Clothier B, Green S, Mills T
1372 (2006) Phytoremediation for the management of metal flux in contaminated sites.
1373 Forest Snow Landsc Res 80:221–234.

1374 Rodríguez RR, Basta NT, Casteel SW, Armstrong FP, Ward DC (2003) Chemical
1375 extraction methods to assess bioavailable arsenic in soil and solid media. J Environ
1376 Qual 32:876–884.

1377 Roy WR, Hassett JJ, Griffin RA (1986) Competitive interactions of phosphate and
1378 molybdate on arsenate adsorption. Soil Sci 142 :203-210.

1379 Saada A, Breeze D, Crouzet C, Cornu S, Baranger P (2003) Adsorption of arsenic (V)
1380 on kaolinite and on kaolinite–humic acid complexes: Role of humic acid nitrogen
1381 groups. Chemosphere 51:757-763.

1382 Sadiq M (1997) Arsenic chemistry in soils: an overview of thermodynamic prediction
1383 and field observations. Water Air Soil Pollut 93:117-136.

1384 Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I (1995)
1385 Phytoremediation: a novel strategy for the removal of toxic metals from the
1386 environment using plants. Biotechnol 13:468-474.

1387 Sasmaz A, Obek E (2009) The accumulation of arsenic, uranium, and boron in *Lemna*
1388 *gibba* L. exposed to secondary effluents. Ecol Eng 35:1564-1567.

1389 Sauvé S (2001) Speciation of metals in soils. In: Allen HE Ed., Bioavailability of
1390 Metals in Terrestrial Ecosystems: Importance of Partitioning for Bioavailability to
1391 Invertebrates, Microbes, and Plants. SETAC Press, Pensacola, Florida, pp. 7-38.

1392 Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-
1393 induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351-1365.

1394 Sharples JM, Meharg AA, Chambers SM, Cairney JWG (2000) Evolution: symbiontic
1395 solution to arsenic contamination. Nature 404:951-952.

1396 Shiowatana J, McLaren RG, Chanmekha N, Samphao A (2001) Fractionation of arsenic
1397 in soil by a continuous-flow sequential extraction method. J Environ Qual 30:1940-
1398 1949.

1399 Signes-Pastor AJ, Deacon C, Jenkins RO, Haris PI, Carbonell-Barrachina AA, Meharg
1400 AA (2009) Arsenic speciation in Japanese rice drinks and condiments. J Environ
1401 Monitor 11:1930-1934.

1402 Simón M, Martín F, García I, Bouza P, Dorronsoro C, Aguilar J (2005) Interaction of
1403 limostone grains and acidic solutions from the oxidation of pyrite tailings. Environ
1404 Pollut 135:65–72.

1405 Smedley PL, Kinninburgh DG (2002) A review of the source, behavior and distribution
1406 of arsenic in natural waters. Appl Geochem 17:517-568.

1407 Smith SE, Read DJ (1997) Mycorrhizal Symbiosis. Academic Press, San Diego,
1408 California.

1409 Smith E, Naidu R, Alston AM (1999) Chemistry of arsenic in soils. I. Sorption of
1410 arsenate and arsenite by four Australian soils. J Environ Qual 28:1719-1726.

1411 Smith E, Naidu R, Alston AM (2002) Chemistry of inorganic arsenic in soils. II. Effect
1412 of phosphorous, sodium, and calcium on arsenic sorption. J Environ Qual 31:557–
1413 563.

1414 Smith SE, Christophersen HM, Pope S, Smith A (2010) Arsenic uptake and toxicity in
1415 plants: integrating mycorrhizal influences. Plant Soil 327:1-21.

1416 Sneller FEC, Van Heerwaarden LM, Kraaijeveld-Smit FJL, Koevoets PLM, Schat H,
1417 Verkleij JAC (2000) Derivatization of phytochelatins from Silene vulgaris, induced
1418 upon exposure to arsenate and cadmium: comparison of derivatization with Ellman's
1419 reagent and monobromobimane. J Agric Food Chem 48:4014–4019.

1420 Srivastava M, Ma LQ, Singh N, Singh S (2005) Antioxidant responses of hyper-
1421 accumulator and sensitive fern species to arsenic. J Exp Bot 56:1335-1342.

1422 Stachowicz M, Hiemstra T, van Riemsdijk WH (2008) Multi-competitive interaction of
1423 As(III) and As(V) oxyanions with Ca^{2+}, Mg^{2+}, PO^{3-}_4, and CO^{2-}_3 ions on goethite. J
1424 Colloid Interf Sci 320:400-414.

1425 Stevenson FJ (1982) Humus Chemistry. Genesis, Composition, Reactions. John Wiley
1426 & Sons, Nueva York.

1427 Su YH, McGrath SP, Zhu YG, Zhao FJ (2008) Highly efficient xylem transport of
1428 arsenite in the arsenic hyperaccumulator *Pteris vittata*. New Phytol 180:434-444.

Takamatsu T, Aoki H, Yoshida T (1982) Determination of arsenate, arsenite, monomethylarsonate, dimethylarsinate in soil polluted with arsenic. Soil Science 133:239-246.

Tassi E, Barbafieri M, Petruzzelli G (2004) Phosphate-Assisted Phytoextraction in As-Contaminated Soil. Eng Life Sci 4:341-346.

Thewys T (2008) Economic opportunities of phytoremediation. In: Bert V Ed, Phytotechnologies in practice: biomass production, agricultural methods, legacy, legal and economic aspects. COST Action 859, Verneuil-en-Halatte, France, pp 45–46.

Thewys T, Kuppens T (2008) Economics of willow pyrolysis after phytoextraction. Int J Phytoremediat 10:561–583

Tomasi N, Weisskopf L, Renella G, Landi L, Pinton R, Varani Z, Nannipieri P, Torrent J, Martinoia E, Cesco S (2008) Flavonoids of white lupin roots participate in phosphorus mobilization from soil. Soil Biol Biochem 40:1971-1974.

Trotta A, Falaschi P, Cornara L, Minganti V, Fusconi A, Drava G, Berta G (2006) Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern *Pteris vittata* L. Chemosphere 65:74–81.

Tripathi RD, Srivastava S, Mishra S, Singh N, Tuli R, Gupta DK, Maathuis FJM (2007) Arsenic hazards: strategies for tolerance and remediation by plants. Trends Biotechnol 25:158–165.

Tu C, Ma LQ (2002) Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. J. Environ. Qual. 31:641-647.

Tu C, Ma LQ (2003) Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions. Environ. Exp. Bot. 50:243-251.

Ultra VU, Tanaka S, Sakurai K, Iwasaki K (2007a) Effects of arbuscular mycorrhiza and phosphorus application on arsenic toxicity in sunflower (*Helianthus annuus* L.) and on the transformation of arsenic in the rhizosphere. Plant Soil 290:29–41.

Ultra VUY, Tanaka S, Sakurai K, Iwasaki K (2007b) Arbuscular mycorrhizal fungus (Glomus aggregatum) influences biotransformation of arsenic in the rhizosphere of sunflower (Helianthus annuus L.). Soil Sci Plant Nutr 53:499–508.

Vanderlinden K, Polo MJ, Ordoñez R, Giráldez R (2006) Spatiotemporal evolution of soil pH and zinc aftet the Aznalcóllar mine spill. J. Environ. Qual. 35:37-49.

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lie D, Mench M (2009)

1464 Phytoremediation of contaminated soils and groundwater: lessons from the field.
1465 Environ. Sci. Pollut. Res. 16:765-794.

1466 Vazquez MD, Barcelo J, Poschenrieder C, Madico J, Hatton P, Baker AJM, Cope GH
1467 (1992) Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae) a
1468 metallophyte that can hyperaccumulate both metals. J Plant Physiol 140:350–355.

1469 Vázquez S, Esteban E, Goldsbrough P (2005) Arsenate-induced phytochelatins in white
1470 lupin: influence of phosphate status. Physiol Plantarum 124:41-49.

1471 Vázquez S, Agha R, Granado A, Sarro MJ, Esteban E, Peñalosa JM, Carpena RO
1472 (2006) Use of white lupin plant for phytostabilization of Cd and As polluted acid
1473 soil. Water Air Soil Pollut 177:349–365.

1474 Vázquez S, Moreno E, Carpena R (2007) Role of iron in the retention of As by cell
1475 walls of lupin roots. COST meeting, Vilnius, Lithuania.

1476 Vázquez S, Moreno E, Carpena RO (2008a) Bioavailability of metals and As from
1477 acidified multicontaminated soils: use of white lupin to validate several extraction
1478 methods. Environ Geochem Health 30:193-198.

1479 Vázquez S, Esteban E, Carpena RO (2008b) Evolution of arsenate toxicity in nodulated
1480 white lupine in a long-term culture. J Agric Food Chem 56:8580-8587.

1481 Vázquez S, Carpena RO, Bernal MP (2008c) Contribution of heavy metals and As-
1482 loaded lupin root mineralization to the availability of the pollutants in multi-
1483 contaminated soils. Environ Pollut 152:373-379.

1484 Verbruggen N, Hermans C, Schat H (2009) Mechanisms to cope with arsenic or
1485 cadmium excess in plants. Current Opin Plant Biol 12364-12372.

1486 Vetterlein D, Szegedi K, Neackermann J, Mattusch J, Neue HU, Tanneberg H, Jahn R
1487 (2007) Competitive mobilization of phosphate and arsenate associated with goethite
1488 by root activity. J Environ Qual 36:1811–1820.

1489 Vithanage M, Senevirathna W, Chandrajith R, Weerasooriya R (2007) Arsenic binding
1490 mechanisms on natural red earth. A pontential substrate for pollution control. Sci Tot
1491 Environ 379:244-248.

1492 Wang S, Mulligan C (2006) Natural attenuation processes for remediation of arsenic
1493 contaminated soils and groundwater. J Hazar Mater 138:459-470.

1494 Warren GP, Alloway BJ, Lepp NW, Singh B, Bochereau FJM, Penny C (2003) Field
1495 trials to assess the uptake of arsenic by vegetables from contaminated soils and soil
1496 remediation with iron oxides. Sci Tot Environ 311:19-33.

1497 Waychunas GA, Fuller CC, Rea BA, Davis JA (1993) Surface chemistry of ferihydrite:
1498 1. EXAFS studies on the geometry of coprecipitated and adsorbed arsenate. Geochim
1499 Cosmochim Ac 57:2251-2269.

1500 Weng L, Van Riemsdijk WH, Hiemstra T (2009) Effects of fulvic acids on arsenate
1501 adsorption to goethite: experiments and modelling. Environ Sci Technol 43:7198-
1502 7204.

1503 Wenzel WW, Kirchbaumer N, Prohaska T, Stingeder G, Lombi E, Adriano DC (2001)
1504 Arsenic fractionation in soils using an improved sequential extraction procedure.
1505 Anal Chim Ac 436:309-323.

1506 Wenzel WW, Brandstetter A, Lombi E, Prohaska T, Stingeder G, Adriano DC (2002)
1507 Arsenic in field-collected soil solutions and extracts of contaminated soils and its
1508 implication to soil standards. J Plant Nutr Soil Sci 165:221-228.

1509 Wenzel WW (2009) Rhizosphere processes and management in plant-assisted
1510 bioremediation (phytoremediation) of soils. Plant Soil 321:385-408.

1511 Williams PN, Price AH, Raab A, Hossain SA, Feldman J, Meharg AA (2005) Variation
1512 in arsenic speciation and concentration in paddy rice related to dietary exposure.
1513 Environ Sci Technol 39:5631-5540.

1514 Williams PN, Islam MR, Adomako EE, Raab A, Hossain SA, Zhu YG, Feldmann J,
1515 Meharg AA (2006) Increase in rice grain arsenic for regions of Bangladesh irrigating
1516 paddies with elevated arsenic in groundwaters. Environ Sci Technol 40:4903-4908.

1517 Williams PN, Villada A, Deacon C, Raab A, Figuerola J, Green AJ, Feldman J, Meharg
1518 AA (2007) Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain
1519 levels compare to wheat and barley. Environ Sci Technol 41:6854-6859.

1520 Woolson EA (1973) Arsenic phytotoxicity and uptake in six vegetable crops. Weed Sci
1521 21:524–527.

1522 Xie ZM, Naidu R (2006) Factors influencing bioavailability of arsenic to crops. In:
1523 Naidu R, et al. Eds. Managing Arsenic in the Environment. From Soils to Human
1524 Health. CSIRO Pub., Collingwood, Australia, pp. 223-234.

1525 Xie QE, Yan XL, Liao XY, Li X (2009) The arsenic hyperaccumulator fern *Pteris*
1526 *vittata* L. Environ Sci Technol 43:8488-8495.

1527 Xu XY, McGrath SP, Zhao FJ (2007) Rapid reduction of arsenate in the medium
1528 mediated by plant roots. New Phytol 176:590–599.

1529 Xu XY, McGrath SP, Meharg AA, Zhao FJ (2008) Growing rice aerobically markedely
1530 decreases arsenic accumulation. Environ Sci Technol 42:5574-5579.

1531 Xu P, Christie P, Liu Y, Zhang J, Li X (2009) The arbuscular mycorrhizal fungus
1532 *Glomus mosseae* can enhance arsenic tolerance in *Medicago truncatula* by increasing
1533 plant phosphorus status and restricting arsenate uptake. Environ Pollut 156:215-220.

1534 Yu Y, Zhang S, Huang H, Luo L, Wen B (2009) Arsenic accumulation and speciation in
1535 maize as affected by inoculation with arbuscular mycorrhizal fungus *Glomus*
1536 *mossaeae*. J Agric Food Chem 57:3695-3701.

1537 Zhang H, Selim HM (2008) Reaction and transport of arsenic in soils: equilibrium and
1538 kinetic modeling. Adv Agron 98:45-115.

1539 Zhang WD, Liu DS, Tian JC, He (2009) Toxicity and accumulation of arsenic in wheat
1540 (*Triticum sestivum* L.) varieties of China. Phyton-Int J Exp Bot 78:147-154.

1541 Zhao FJ, Wang JR, Barker JHA, Schat H, Bleeker PM, McGrath SP (2003) The role of
1542 phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata*. New
1543 Phytol 159:403–410.

1544 Zhao FJ, Ma F, Meharg AA, McGrath SP (2009) Arsenic uptake and metabolism in
1545 plants. New Phytol 181:777-794.

1546 Zhu YG, Rosen BP (2009) Perspectives for genetic engineering for the
1547 phytoremediation of arsenic-contaminated environments: from imagination to
1548 reality? Current Opin Biotechnol 20:220-224.

1549 Zornoza P, Vázquez S, Esteban E, Fernández-Pascual M, Carpena RO (2002)
1550 Cadmium stress in nodulated white lupin: strategies to avoid toxicity. Plant Physiol
1551 Biochem 4:1003–1009.

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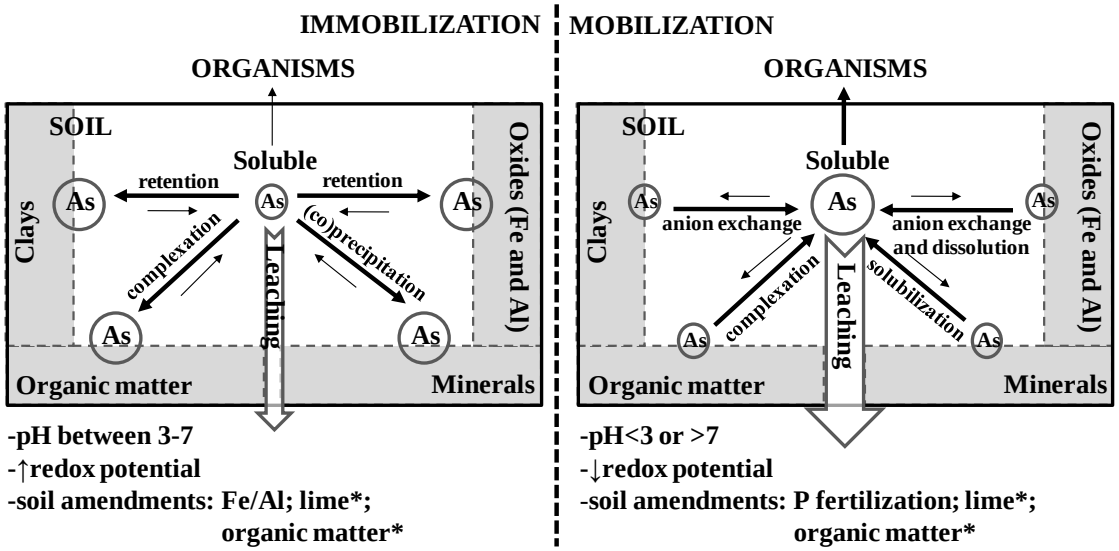
Fig. 1 Soil-As interactions and strategies to manage As availability and mobility in soils

* denotes liming and organic matter application, which may cause either As fixation or release depending on the case

Fig. 2 Pant's traits and physiological mechanisms recognized to be associated with contrasting patterns of As phytoaccumulation: accumulator plants (left) vs. exclusory plants (right). (Blank boxes denote lack of information)

Fig. 3 Optimizing arsenic management in soil-plant systems. (The size of circles and of "As" symbols represent the concentration magnitude of the metalloid. The discontinuous polygons represent the flux of As, with the broadest, being the most intense flux. The white discontinuous squares represent the endpoint of the biomass. In grain, As in organic form ensures the lower ecotoxicologial risk)


IMMOBILIZATION
MOBILIZATION
ORGANISMS
ORGANISMS
SOIL
SOIL
Soluble
Soluble
Clays
Oxides (Fe and Al)
Clays
Oxides (Fe and Al)
As
retention
retention
complexation
(co)precipitation
Leaching
anion exchange
anion exchange and dissolution
complexation
solubilization
Organic matter
Minerals
Organic matter
Minerals
-pH between 3-7
-↑redox potential
-soil amendments: Fe/Al; lime*; organic matter*
-pH<3 or >7
-↓redox potential
-soil amendments: P fertilization; lime*; organic matter*

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PLANT ACCUMULATION	PROCESSES	PLANT EXCLUSION
	Phloem transport	Low phloem As concentration and poor uptake by grains
In shoots	Vacuole storage	In roots
Free As III or V are highly translocated	Xylem transport	Low xylem As concentration, in As-SH complex forms
Low rates of complexation	Complexation	High rates of complexation and accumulation in roots
Very low	Root efflux	Intense
High numbers of membrane transporters, with high affinity for As Low levels of Si or P in the growing media	Uptake	Transporters having higher affinity for P or Si than for As High levels of Si or P in the growing media
As-mobilizing populations	Microbial activity in the rhizosphere	As-immobilizing populations

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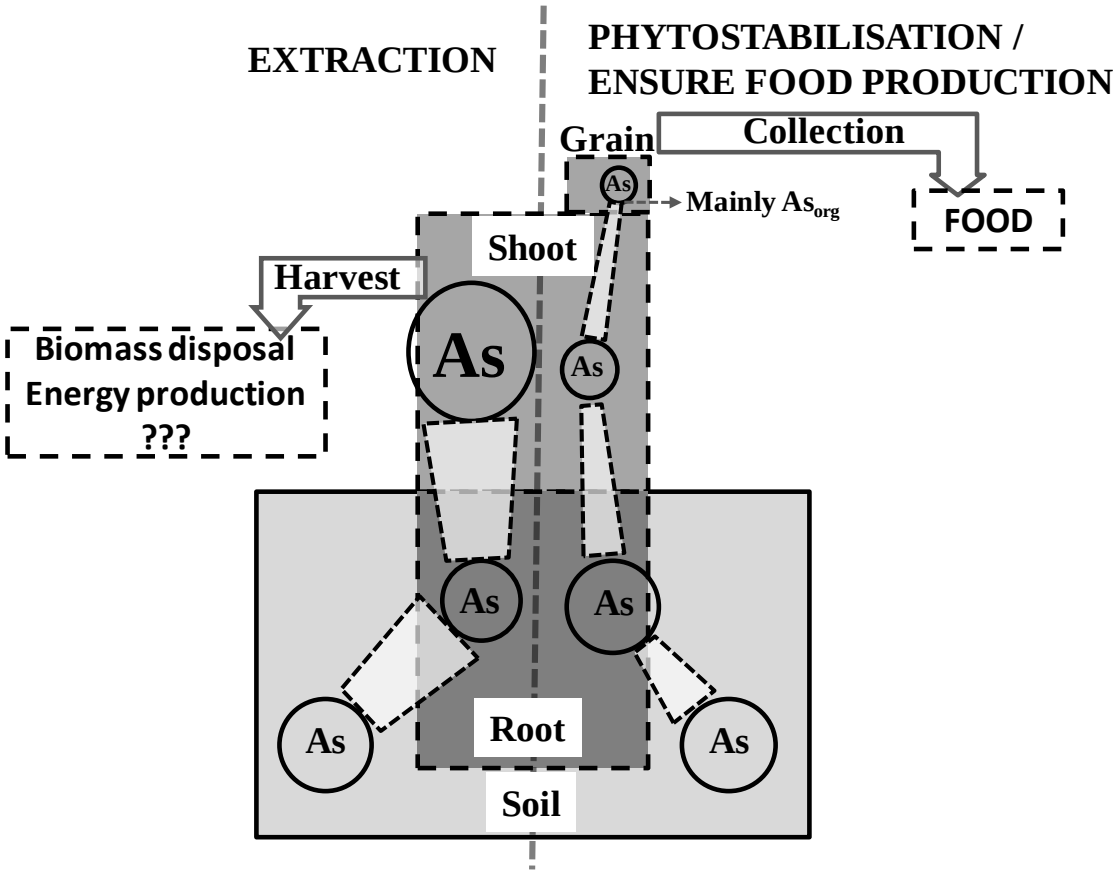
EXTRACTION
PHYTOSTABILISATION /
ENSURE FOOD PRODUCTION
Grain
Collection
As
Mainly As_{org}
FOOD
Shoot
Harvest
Biomass disposal
Energy production
???
As
As
As
As
As
As
Root
Soil

1572

1573